

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 129

MAY 1, 1940

No. 2

RADIOACTIVE PHOSPHORUS AS A TRACER IN ANAEROBIC MUSCULAR CONTRACTION

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Received for publication December 20, 1939

In contraction of striated muscle under anaerobic conditions, the principal chemical reaction is the conversion of glycogen to lactic acid. There is also a decrease in the amount of phosphorus present as phosphocreatine, with corresponding increases in the amounts present as inorganic phosphate and hexosemonophosphate.

Analysis of the possible intermediate stages in these reactions, by means of studies of the enzyme systems of cell-free muscle extracts, has led to a formulation in which these phosphorus changes are regarded as integral parts of the series of reactions by which lactic acid is formed. In this formulation, generally known as the Embden-Meyerhof schema, it is considered that the initial reaction is the formation of hexosemonophosphate from glycogen and inorganic phosphate. This substance then receives an additional phosphate group from adenosine triphosphate; the adenylic acid remaining is re-converted to the original substance by reaction with phosphocreatine. The creatine is re-phosphorylated at a later stage in the process. From this it can be seen that an atom of P present in any one of the four forms may readily be converted to any one of the others in the course of the transformations.

In the summary of the Embden-Meyerhof schema given by Needham (6) it is pointed out that there are some discrepancies between the phosphorus changes observed in anaerobic contraction and the requirements of this reaction cycle. There is no satisfactory explanation for the decrease in phosphocreatine content, for any creatine liberated early in the cycle should acquire a phosphate group by a subsequent reaction. There is no basis in the known reactions of extracts for the increased hexosemonophosphate content which is observed, and a decreased inorganic phosphate content is to be anticipated, rather than the increase usually found.

Another theory has been proposed concerning the relations in anaerobic contraction (8). According to this formulation, the phosphorus changes are not directly concerned with the formation of lactic acid. The hydrolysis of phosphocreatine serves to supply alkali for the neutralization of the lactic acid, and the formation of hexosemonophosphate, from phosphocreatine P rather than from inorganic P, is considered to be a supplementary source of energy for contraction in the absence of oxygen, used whenever the lactic acid forming mechanism becomes inadequate.

The over-all changes in the contracting muscle do not, of course, give any indication of the possible intermediate stages. However, the use of radioactive phosphorus as tracer offers the possibility of determining whether the formation of lactic acid in contraction does involve the phosphate interchanges postulated by the Embden-Meyerhof schema. Many of these interchanges have been shown to take place in extracts in which radiophosphorus was present (2, 5), but this positive finding does not have any direct bearing on the question of whether they take place within the intact cell. It does, however, emphasize that the chemical properties of the radioactive isotope are the same as those of the ordinary stable isotope.

For the tracer method to furnish data which are of critical value, it is necessary that the normal metabolic processes result in a differential distribution of the labeled atoms among the phosphorus compounds of the resting muscle. The observations of Hevesy and Rebbe (3) indicate that this may not hold as between inorganic and phosphocreatine P in frogs, and those of Korzybski and Parnas (4) indicate that this may not be the case with respect to inorganic and adenosine triphosphate P in rabbits. In the present experiments, however, in which much larger amounts of the radioactive isotope were used than were available to these investigators, a definite differential distribution was found. As can be seen from the values for the relative radioactivities of the various fractions in the resting muscles, in table 1, the inorganic phosphate had ten times the concentration of the radiophosphorus that was present in the phosphocreatine fraction. This, in turn, generally had a higher concentration than the readily hydrolyzable P of the adenosine triphosphate. The hexosemonophosphate P had the lowest concentration of the radiophosphorus. The situation was therefore favorable for the study of the effect of lactic acid formation on the possible interchanges.

Since the Embden-Meyerhof schema postulates at least one complete cycle of interchanges between the four P compounds in the formation of lactic acid, it can be seen that the formation of any appreciable amount of lactic acid by these reactions should result in a considerable redistribution of the radiophosphorus. It can be calculated that the formation of one molecule of lactic acid for each two atoms of P present in any one of the compounds should result in practically uniform distribution of the radio-

phosphorus, if the reactions take place within the intact muscle cell in the same way as they do in the cell-free extracts. Resting muscles of cats contain about 1 millimol per 100 grams of P in the form of the readily hydrolyzable groups of adenosine triphosphate; inorganic phosphate and

TABLE 1

Distribution of radiophosphorus among the different compounds in resting and stimulated gastrocnemius muscles of cats

Stimulation consisted of 15 seconds' isometric tetanus.

Values are expressed in terms of relative radioactivity per milligram of P, referred to the inorganic P of the resting muscle as 100.

R, resting muscle; S, stimulated muscle.

EXPERIMENT NO.		INORGANIC P	PHOSPHO-CREATINE P	READILY HYDROLYZED P OF ADENOSINE TRIPHOSPHATE	HEXOSEMONO-PHOSPHATE P
1	R	100	10	17	1.5
	S	46	9.5	13.8	4.6
2	R	100	11.5	10.1	7.7
	S	72	10.1	9.6	9.1
3	R	100	9.2	6.4	
	S	87.5	13.0	9.0	
4	R	100	6.6	4.4	
	S	91.5	8.1	6.1	
5	R	100	9.8	6.7	3.5
	S	75	8.1	8.8	6.7
6	R	100	16.5	10	0.8
	S	70	16.3	7.5	4.8
7	R	100	8.9	4.7	1.2
	S	96	10.1	7.7	4.2

Effect of 15 seconds, isometric tetanus on P distribution; values expressed as milligrams per cent of P

1						LACTIC ACID
1	R	14	60	33	7	8
	S	33	29	31	25	106

hexosemonophosphate are present in smaller amounts, and phosphocreatine in somewhat larger concentration. On this basis, the formation of less than one millimol of lactic acid per 100 grams of muscle should result in uniform distribution of the radiophosphorus.

On the other hand, if only those changes in phosphorus distribution took

place which are observed on balance, i.e., the hydrolysis of some phosphocreatine, and the conversion of some P from this form to hexosemonophosphate, the stimulated muscle should show an entirely different distribution of the radiophosphorus among the four compounds. Since some phosphocreatine remains, and since there is no change in the adenosine triphosphate, the P present in these forms in the stimulated muscle should show the same relative radioactivities with respect to each other as in the resting muscle. The hexosemonophosphate P of the stimulated muscle should show an increase in the concentration of the radiophosphorus to a point not in excess of the value for the phosphocreatine P. The inorganic phosphate of the stimulated muscle should have a relative radioactivity intermediate between the values for the inorganic and phosphocreatine P of the resting muscle. In other words, the extract formulation requires that the formation of lactic acid in contraction result in the loss of the differential distribution of the radiophosphorus, whereas the other hypothesis requires that the stimulated muscle retain the differential distribution.

The radiophosphorus used was supplied by the Department of Physics of the University of Michigan. It was produced by bombardment of iron phosphide with deuterons in the cyclotron. The iron phosphide was then fused with sodium peroxide. The P was thus converted to trisodium phosphate. This was isolated, dissolved in water, and converted to the disodium salt by the addition of the calculated amount of hydrochloric acid.

The experiments were performed on cats under amytal anesthesia. After anesthetization, the solution containing the radiophosphorus was injected subcutaneously. About 0.1 millicurie was used for each experiment. It can be calculated from the data of Crane (1) that this amount of radioactive material is less than one one-thousandth of the amount needed to give an erythema dose over the period of exposure. The results are therefore not affected by the general biological effects of the radiation.

The gastrocnemius muscles were prepared in the usual way. Two hours after the injection of the radioactive material one gastrocnemius was subjected to 15 seconds of isometric tetanus, and then frozen. The resting muscle was then frozen. This stimulation period was used because it had been found previously that it resulted in the formation of one millimol or more of lactic acid per 100 grams of muscle.

Trichloroacetic acid filtrates were prepared, using 5 cc. of 10 per cent solution per gram of muscle. One portion of the filtrate was treated with magnesia mixture made up with magnesium and ammonium nitrates, and from this portion were separated the P present as inorganic phosphate, phosphocreatine, and the readily hydrolyzed fraction of adenosine triphosphate. Another portion was neutralized to phenolphthalein with barium hydroxide, for the isolation of the hexosemonophosphate P.

The first portion was let stand overnight, and the precipitate of magnesium ammonium phosphate separated by filtration, dissolved in dilute nitric acid, and the P precipitated as phosphomolybdate. Nitric acid was added to the ammoniacal filtrate to bring the acidity to 0.2 N, and ammonium molybdate solution added. The whole was let stand $1\frac{1}{2}$ hours at refrigerator temperature to hydrolyze and precipitate the phosphocreatine P. The filtrate from this fraction was acidified with more nitric acid, to 0.5 N, heated to boiling, and kept at this temperature for 15 minutes, to hydrolyze the adenosine triphosphate. The solution was then cooled, the acidity reduced to 0.2 by the addition of ammonia, and the precipitate separated by filtration. The precipitate was freed from molybdic acid by solution in dilute ammonia and reprecipitation with nitric acid.

The portion of the filtrate treated with barium hydroxide was freed from the precipitated barium phosphate and adenosine triphosphate by centrifugation. The supernatant fluid was treated with nitric acid and ammonium molybdate, and let stand to hydrolyze and precipitate the phosphocreatine P. The precipitate was separated by filtration, the precipitate discarded, and the filtrate evaporated to a small volume after the addition of concentrated nitric acid. The addition of nitric acid and the evaporation were repeated, to complete the hydrolysis of the hexosemonophosphate. The residue was taken up in water, the precipitate separated by filtration, and freed from molybdic acid by solution in ammonia and precipitation with nitric acid.

The separate treatment of the two portions of muscle filtrate was necessary because no single reagent is satisfactory for the complete separation of the four phosphorus compounds. When barium hydroxide is used to precipitate inorganic phosphate and adenosine triphosphate, considerable phosphocreatine is adsorbed on the bulky precipitate, and is thus recorded as inorganic phosphate. Also, the solubility of barium phosphate is appreciable, so that some is carried over into the phosphocreatine fraction. Attempts to precipitate adenosine triphosphate by barium after magnesium mixture precipitation of the inorganic phosphate, were not successful.

The various phosphomolybdate precipitates were dissolved separately in dilute ammonia, and the solutions evaporated to dryness on the steam bath. Each residue was taken up in a small quantity of water; the major portion of this was used for the determination of the radioactivity, and a small aliquot was used for colorimetric determination of P. The portion used for determination of radioactivity was transferred to a 50 mm. watch glass, and the solution evaporated to dryness on the steam bath. Each watch glass was wrapped in cellophane, with a single thickness over the concave side. The cellophane wrapping served to protect the electrometer chamber from having any radioactive material fall upon it from an inverted watch glass.

Measurements of the rate of ion production, from which the relative radioactivities were calculated, were made with an Edelmann electrometer. All the samples from one experiment were measured at one time, thus making it unnecessary to correct for decay. The watch glasses were all placed in the same position relative to the ionization chamber. The least active material, representing the hexosemonophosphate of the resting muscles, produced ions at two to three times the background rate; these measurements are accurate only to within 10 or 15 per cent. The most active material, the inorganic phosphate fractions, produced ionization at twenty to sixty times the background rate. In this range, the measurements are accurate to within one or two per cent. In the table, the results are given in terms of relative ionization rate per milligram of P, referred in each case to the inorganic P of the resting muscle as 100.

The results of the experiments are given in table 1. It can be clearly seen that the differential distribution of the radiophosphorus which is present in the resting muscles, is retained through the stimulation period. The findings correspond to the requirements of the hypothesis derived from observations on the contracting muscles, and do not correspond in any particular to the distribution which would result if the formation of lactic acid had taken place by way of the reactions of the Embden-Meyerhof cycle. It is therefore evident that this schema of reactions does not describe the mechanism of lactic acid formation used by the contracting muscle.

The results show that adenosine triphosphate does not act as phosphate transporter in the formation of lactic acid. The question of the function of this compound in contraction still remains open.

The finding that phosphocreatine does not act as a transporter of phosphorus in the formation of lactic acid lends support to the hypothesis (8) that the function of the hydrolysis of this substance is to supply alkali to neutralize the lactic acid formed in anaerobic contraction, and thus prevent change in pH within the fiber.

It is also seen that the P for the formation of hexosemonophosphate is derived directly from phosphocreatine, and not from inorganic phosphate. In addition, it is evident that this hexosemonophosphate does not undergo any further change during anaerobic contraction. These observations are in support of the conclusions drawn from earlier observations on normal (7) and iodoacetate-poisoned (8) muscle, that the formation of this substance is a supplementary source of energy under anaerobic conditions whenever the lactic acid forming mechanism is inadequate.

SUMMARY AND CONCLUSIONS

1. Radioactive phosphorus has been used to study the changes in phosphorus distribution which accompany the formation of lactic acid in contraction of striated muscle under anaerobic conditions.

2. The "labeled" atoms undergo a differential distribution among the phosphorus compounds present in resting muscle.

3. This differential distribution is retained in the course of formation of lactic acid in tetanic contraction. The changes found correspond to the net changes in phosphate distribution which have been reported previously. These consist of the hydrolysis of part of the phosphocreatine, and the reaction of another part with glycogen to form hexosemonophosphate.

4. No evidence has been found that the interchanges of phosphate groups postulated by the Embden-Meyerhof schema take place in contracting muscle. It is therefore concluded that this set of reactions does not represent the mechanism by which lactic acid is formed within the muscle cell.

5. Neither phosphocreatine nor adenosine triphosphate takes part in the formation of lactic acid within the muscle cell.

6. The evidence obtained by the use of radiophosphorus supports the hypothesis that the function of phosphocreatine hydrolysis in contraction is to supply alkali for the neutralization of lactic acid, and that the formation of hexosemonophosphate is a supplementary source of energy under anaerobic conditions whenever the lactic acid mechanism is inadequate.

The writer wishes to express his appreciation to the Horace H. Rackham Fund for support of the operations of the cyclotron, and to Prof. S. A. Goudsmit, Prof. J. M. Cork, Dr. J. L. Lawson, and Mr. W. H. Sullivan of the Department of Physics of the University of Michigan for their generous assistance.

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CYCLIC PENETRABILITY OF HUMAN CERVICAL MUCUS TO SPERMATOZOA IN VITRO

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Received for publication December 29, 1939

Early in 1939, in connection with *in vivo* spermatozoan migration studies carried on in collaboration with Dr. C. S. Stevenson, a new technique for studying the penetrability of cervical mucus *in vitro* was devised by one of us (J. K. L.). The new technique was worked out as a control measure for the *in vivo* experiments, because serious defects were encountered when the original method of Miller and Kurzrok (1932) was tried.

Miller and Kurzrok had noted that cervical mucus sometimes formed an impenetrable barrier to spermatozoa, and this mucus they called "infected." Séguy and Vimeux (1933) suggested that cervical mucus undergoes a cycle in which, at about the time of ovulation, the mucus becomes more abundant, freer of leucocytes, and temporarily permeable to spermatozoa. Because of the general failure of reviewers in this country to recognize the work of Séguy and Vimeux, and because of the availability of a new, and perhaps better method of studying spermatozoan migration through cervical mucus *in vitro*, a re-investigation of the phenomenon of cyclic penetrability was decided upon.

MATERIALS AND METHODS. Cervical mucus was collected from women by means of a sterile dry speculum and a long glass pipette. Isolated specimens were obtained from the Birth Control and Dystocia Clinics of the Johns Hopkins Hospital, and through the coöperation of private physicians. In all, the cervical mucus of fifty-eight women showing normal cycles has been studied; six of these individuals have been seen more than once, and five studied for at least one cycle. One case was followed for three complete cycles.

An attempt was made to correlate the amount of mucus secreted, its pH, the number of leucocytes contained, and the longevity of spermatozoa in the mucus, with its penetrability by spermatozoa.

Mucus was judged to be 1, scant; 2, moderate, or 3, abundant at the time of collection. Relative viscosity was determined by the amount of force needed to draw the mucus into capillary tubes. Two electrometric

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methods and one colorimetric method of determining pH were tried. The colorimetric method (nitrazine paper, Squibb) was finally used routinely. Checks of this method against the quinhydrone electrode were satisfactory. The relative number of leucocytes was determined by microscopic examination, as was the longevity of motility of the spermatozoa in or near the mucus.

The penetrability of cervical mucus to spermatozoa³ was studied as follows: Capillary tubing of 0.10 to 0.40 mm. inside diameter was prepared. A small amount of the mucus was drawn into a length of tubing and the relative viscosity was observed. After the mucus a small bubble of air was drawn up, then a column of fresh semen, and the time when this was done was recorded. The purpose of the bubble was to prevent mechanical mixing of semen and mucus during their arrangement in the capillary tube. If the bubble was of correct size this was accomplished and still enough mucus adhered to the tube walls to furnish a medium through which spermatozoa could pass the bubble and begin attacking the mucus. Several satisfactory tubes were prepared and sealed with mineral oil. The attack of the spermatozoa on the mucus was watched through the microscope. If penetration occurred the bubble furnished a landmark, and, by using a stop watch and a calibrated mechanical stage, the rate of penetration of a single spermatozoön could be measured accurately.

The tubes were set aside, along with control tubes of semen, for occasional examination to determine motility survival time of the spermatozoa, in semen and in the mucus.

RESULTS. It was noted from the first that some samples of cervical mucus could be penetrated by spermatozoa, others could not. It was soon observed that mucus exhibiting penetrability came from women at or near the mid-cycle, that is, near the expected time of ovulation.

A complete exploration of the cycle for normal women, aside from one case, was made by plotting the rate of penetration per unit time against the time of the cycle (lower curve, fig. 1). This one case, X, followed through three cycles, was plotted in figure 2. The curves in figure 1 have been smoothed by mathematical calculation on a running average basis. A study of the lower curves in both figures tells approximately the same story.

Examination of figure 1 shows that during the menstrual flow there was relatively easy penetration of the menstrual discharge by spermatozoa.

Soon after the menstrual flow ceased, however, the penetrability of the cervical mucus decreased. In only two samples out of nine studied between days four and eight was there any evidence of penetration by spermatozoa. The two cycles of case X studied during this part of the cycle show also that no penetration occurred in this part of the cycle.

Then came a period during which many samples of cervical mucus

³ The usual sources of spermatozoa for these experiments were two donors with good sperm counts and good sperm motility.

showed penetrability (20 samples out of 29). The duration of this period was from about day nine through day 19, with a maximum at day 14-15. During this peak time spermatozoa sometimes raced through the mucus at the rate of 2 to 3 mm. per minute.⁴

Case X (fig. 2) showed peaks of penetrability on days 15, 10-13, and 11. In the first cycle a sample taken on day 14 (day of Mittelschmerz) showed

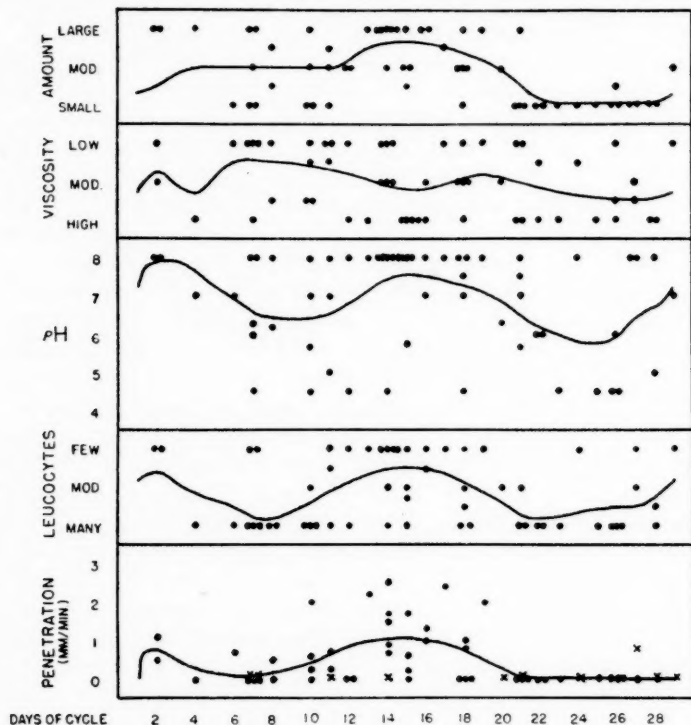


Fig. 1. Relation between the amount of cervical mucus, its viscosity, pH, number of leucocytes contained, and penetration (mm./min.) by spermatozoa and the time in the menstrual cycle. These curves are based on observations on 57 women.

a penetration of 1 mm. per minute. The next day the penetrability averaged 3 mm. per minute. On day 16 it had dropped to 1 mm. again, and on day 17 spermatozoa would not penetrate the cervical mucus at all. Penetrability ceased in the second cycle by day 15 and in the third cycle by day 18.

⁴ Spermatozoa move about in semen with a wide lashing of the tail. In penetrable mucus, however, the lashings are faster, with less amplitude, and the forward motion achieved seems greater.

Finally, there came a period of complete impenetrability, during which the mucus presented an impervious barrier to the spermatozoa. This part of the cycle lasted from about day 20 to the next menstruation. It is striking to note that of the 18 observations during this part of the cycle plotted in figure 1 only one case showed penetration past a fraction of a millimeter, and in this sample spermatozoa were stopped (immobilized)

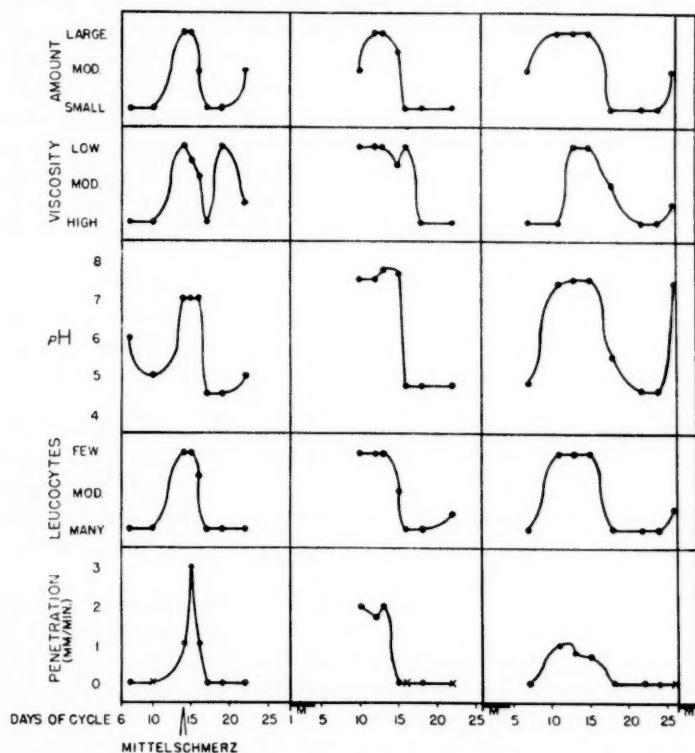


Fig. 2. Relation between the amount of cervical mucus, its viscosity, pH, number of leucocytes contained, and penetration (mm./min.) by spermatozoa and the time in the menstrual cycle. These curves are based on observations on one individual for three successive cycles.

after penetrating about 1 millimeter. Figure 2 shows that for three consecutive cycles of case X the period of impenetrability began on days 17, 15, and 18, respectively. No penetration was seen after this part of the cycle began.

An attempt was made to ascertain what factor or factors in the properties of the cervical mucus might be correlated with and perhaps be responsible for this cyclic variation.

Investigation of the relative number of leucocytes in the cervical mucus revealed an inverse correlation with penetrability. Figure 1 shows that during the menstrual flow, when leucocytes are scarce but erythrocytes numerous, and during the mid-cycle, when leucocytes are also scarce, there is a good chance of spermatozoa penetrating the mucus, whereas during the part of the cycle just after menstruation, and again between mid-cycle and the following menstruation, when the leucocytes are closely packed in the mucus, spermatozoa stand little chance of penetrating far. An almost perfect correlation between penetrability of mucus and scarcity of cells in it is seen in the three cycles plotted for case X in figure 2.

That this is not the only factor correlated with penetrability is indicated by those cases during the last part of the cycle, plotted in figure 1, in which spermatozoa began to migrate (marked by X) but were soon immobilized. In most of these cases the mucus contained but few leucocytes, yet the spermatozoa ended a short invasion by lashing slowly and soon stopping altogether.

A study of the relative acidity or alkalinity of the cervical mucus shows a positive correlation of pH with penetrability. During the menstrual flow, when the discharge is alkaline due to contained blood, and during the mid-cycle, when the pH is again high, the mucus is, in general, penetrable to spermatozoa. On the other hand, during the part of the cycle between menstruation and mid-cycle, and again between mid-cycle and the subsequent menses, when the cervical secretion is generally acid, penetrability is at a low ebb. The conclusion is borne out by data plotted in both figures. Here again it cannot be said that because a sample of mucus is alkaline it need be penetrable to spermatozoa. Experience shows that pH is not the sole factor.

Observations on the relative viscosity by our methods with relation to penetrability show no striking correlation in either direction. It is true that in the three cycles plotted for case X (fig. 2), there seems to be a fair correlation between low viscosity and penetration, but an examination of figure 1 casts doubt upon the idea.

It can be gleaned from both figures that the relative amount of cervical mucus in relation to its penetrability shows a good correlation between a large amount of secretion and penetration. It cannot be said that there is a large amount of mucus during the menstrual flow, but the quantity of discharge is large. Likewise the amount of mucus secreted during the mid-cycle is generally large. A maximum occurs at about day 14 of the cycle, sloping off in both directions, particularly toward the end of the cycle, during which mucus is usually very scant.

Figure 3 represents the relationship between the phase of the menstrual cycle and the survival of spermatozoa in the mucus *in vitro*. It will be seen that survival past one or two hours was rare in mucus from women in

the phase between menstruation and mid-cycle. Survival was best at about mid-cycle and fell to a very low level in the phase between mid-cycle and the following menstruation. In the one case in which survival of spermatozoa in the menstrual discharge was studied, motility persisted for a period of 28 hours, which is well above average, even for mid-cycle mucus. It was only in the mid-cycle that a few cases were found in which spermatozoa survived longer in the mucus than they did in the control tubes of semen, or in the semen portion of the semen-mucus tubes.

Interest in the idea of periodic or cyclic penetrability of cervical mucus by spermatozoa in the normal cycle led to a few observations on cervical mucus of women otherwise normal, but not undergoing cycles because of lactation. Of six women amenorrheic because of lactation (six weeks to twenty-one months post-partum), only one had mucus penetrable by

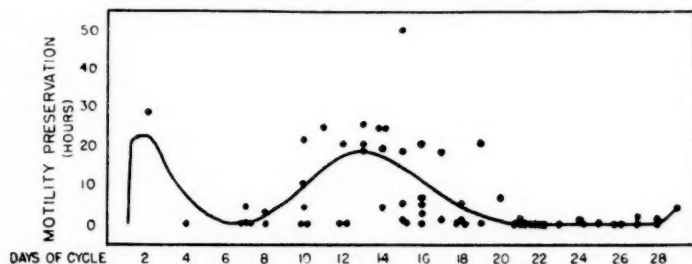


Fig. 3. Relation between motility preservation in hours and the time in the menstrual cycle in days. Each point represents the average of several determinations on one sample of mucus.

spermatozoa. This woman was weaning her baby. The small number of cases precludes a general statement at the present time.

DISCUSSION. Our results confirm the claim of Séguy and Vimeux (1933) that cervical mucus undergoes a cycle of permeability, although the methods used were not the same. The French authors did *in vivo* studies as well as slide and cover glass preparations.

We believe our micro-tube method, with the barrier-marker bubble, to be the *in vitro* method of choice. It eliminates the possibility of undiscoverable mechanical mixing. It prevents drying of the preparation, with the attendant evils of currents in the mucus and makes possible a quantitative measure of penetration when this occurs, desiderata not attained in the slide-cover slip method of Miller and Kurzrok.

We do not share the opinion of Séguy and Vimeux that the cervical mucus is always alkaline. We think, rather, with Palmer and Michon-Adjonbel (1938), that the reason there is a correlation between abundance and alkalinity of cervical mucus is this: The acid vaginal contents always

tend to neutralize and acidify the adjacent cervical mucus. At the mid-cycle, when a large amount of glairy mucus is secreted, probably under the influence of estrogens (Séguy and Simonnet, 1933, and Wollner, 1938), the acidified cervical mucus is pushed into the vagina and no effective barrier is formed at the external os. On the other hand, at the stages in the cycle when cervical mucus is not secreted so rapidly, the acidified mucus is not pushed along by fresh mucus, but remains in place, thus forming a barrier against spermatozoan invasion. Undoubtedly the acidity is not the only factor making for impenetrability, but it has such a deleterious effect on the spermatozoa that they are soon immobilized.

Rate of secretion probably bears a relation to abundance and glairy translucence of the cervical mucus. Rapid secretion would produce a large amount of mucus with few leucocytes, whereas a slow secretory rate would result in scant mucus filled with leucocytes.

It is suggested that viscosity may play a much larger part in the penetrability of cervical mucus than our results indicate. In fact, there seems to be some correlation between low viscosity and penetrability in figure 2 although this idea is not borne out in figure 1. It is admitted that any approximation of the relative viscosity by our methods was bound to be inexact, and it does not follow that the measurable viscosity is at all a criterion of the ease of penetration by a spermatozoön. It is suggested that the presence of large numbers of leucocytes, or other particles of similar size, might cause the measurable viscosity to be lower, without affecting the viscosity of the fluid between the cells. It is, of course, this fluid which the spermatozoa must traverse.

There would seem to be several practical implications of this investigation. A use may be found for this method of studying the penetrability of cervical mucus in cases of unexplained sterility. Schoekaert and Delrue (1938) have suggested the importance of the pH of cervical mucus in such cases, and Kleegman (1936) has suggested that artificial insemination has a field of usefulness where a cervical secretion which blocks the ascent of spermatozoa cannot be corrected. Of course, all other possible causes of sterility should be eliminated before recourse to a study of the cervical secretions.

SUMMARY

1. A new method of studying penetration of cervical mucus by spermatozoa *in vitro* is described, involving the use of micro-tubes and barrier-marker bubble.

2. This method is considered better than slide-cover glass preparations because it prevents undiscoverable mechanical mixing of mucus and semen and drying of the preparation, and because it can be used to study penetration quantitatively and to study longevity of the spermatozoa in the mucus.

3. Results by this method confirmed the *in vivo* and *in vitro* findings of Séguy and Vimeux, namely, that there is a cyclic production of a glairy, translucent cervical mucus at about mid-cycle, which renders the cervix temporarily penetrable by spermatozoa. Roughly, the spread of this phase was, in this study, from day 9 through 19, but for any one cycle this period may be restricted to four days or so.

4. Indications are that menstrual discharge is penetrable by spermatozoa, but the mucus of the phase between menstruation and the mid-cycle is relatively impenetrable, and that of the phase between mid-cycle and the subsequent menstruation is impenetrable.

5. Correlated with the mid-cycle peak in penetrability is the peak in freedom from leucocytes, the peak in pH (except during the menstrual flow), the peak in the amount of mucus secreted, and the height of conditions favoring greatest longevity of spermatozoa in the mucus. It may well be that the actual viscosity of the mucus encountered by spermatozoa is lowest at the mid-cycle. It is suggested that both low pH and large number of cells in the "secretory phase" of the menstrual cycle may be caused, at least in part, by the low rate of secretion of the mucus.

6. An investigation of the cervical mucus over the cycle, by the *in vitro* method, is suggested in cases of otherwise unexplained sterility. A field of usefulness for artificial insemination may be developed in sterilities due to a persistent impenetrable cervical mucus.

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THE EFFECT OF RENAL INSUFFICIENCY ON THE RESPONSE OF SERUM CALCIUM AFTER ADMINISTRATION OF PARATHYROID HORMONE IN THE RAT

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Received for publication January 5, 1940

A relationship between renal disease and the parathyroid glands has been established by numerous investigators in both man and experimental animals. This subject has been reviewed by Anderson (1) and by Pappenheimer (2). It has been pointed out that *a*, parathyroid hyperplasia is proportional to the degree of kidney damage in patients; *b*, renal damage is frequently associated with hyperparathyroidism in patients; *c*, the parathyroids are enlarged in cases of renal rickets; *d*, partial nephrectomy in rats leads to an increase in the size of the parathyroid glands; and *e*, complete nephrectomy protects the dog from the effect of large doses of parathyroid hormone.

This paper describes the changes in serum calcium after injection of parathyroid extract in the intact and partially nephrectomized rats. The results of these experiments indicate that renal insufficiency produced by partial nephrectomy diminishes the response normally produced by the parathyroid hormone.

METHODS. White rats of Wistar strain were used as experimental animals. Partial nephrectomy was performed on male animals according to the procedure described by Chanutin and Ferris (3). The animals were fed on a stock diet (Bal Ra).² About six months after operation the animals were either injected with saline or parathyroid extract. Intact male rats of the same age served as controls. The procedure of Pugsley (4) was used in which either 1 cc. (20 units) or 2 cc. (40 units) of Parathormone (Lilly) was injected subcutaneously on two successive days. On the third day the animals were anesthetized with ether and blood was withdrawn from the abdominal aorta with a needle and syringe. The serum was analyzed for calcium according to the procedure recommended by Sobel and Sklersky (5) and phosphorus by the method described by Bodansky (6). In most instances there was not enough serum for more than one determination.

¹ Supported by a grant from the John and Mary R. Markle Foundation.

² Bal Ra purchased from the Valentine Meat Juice Company of Richmond, Va.

RESULTS. The individual data and the respective average values are presented in figure 1. The results for the control intact and partially nephrectomized animals are almost identical, yielding average values of 10.1 and 10.2 mgm. per cent calcium. The intact animals respond to parathormone injection with average values of 13.1 and 15.5 mgm. per cent calcium after two injections of 20 and 40 units, respectively. These results are similar to those reported by Pugsley (4). The partially nephrectomized rats respond only to a slight extent with mean values of 11.6 and 11.0 mgm. per cent calcium after injection of 20 and 40 units of parathormone, respectively. There is no significant statistical difference between these two mean values. Of the seventeen values obtained for serum calcium in partially nephrectomized rats, six values were within

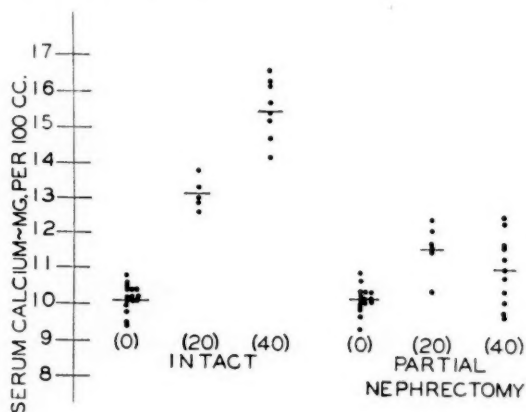


Fig. 1. Individual values for serum calcium in intact and partially nephrectomized rats after subcutaneous injection of parathyroid extract. Figures in parentheses represent units of parathormone injected on 2 successive days.

the normal control range. Representative values for total solids of serum showed no change from the normal as a result of injection of parathyroid extract.

A few analyses of serum inorganic phosphorus in the partially nephrectomized animals were done. The values for two uninjected animals were 6.6 and 6.2 mgm. per cent P, respectively, and 8.8, 8.0 and 6.8 mgm. per cent P for three rats injected with parathyroid extract.

The poor response of the partially nephrectomized rat to the parathyroid hormone must be associated with the primary reduction of functioning kidney tissue. Donohue, Spingarn and Pappenheimer (7) demonstrated that the hyperparathyroidism resulting from the renal insufficiency produced by partial nephrectomy is responsible for an increased deposition of

calcium in the residual kidney tissue. Furthermore, Tweedy and his co-workers (8) (9) have shown that complete nephrectomy prevents the mobilization of blood calcium after parathyroid extract administration. It is probable that the inability to excrete phosphates is an important determining factor in explaining the results obtained in the partially nephrectomized rat. Further evidence has been presented in this paper to show a relationship between the parathyroids and the kidneys.

SUMMARY

Renal insufficiency produced by partial nephrectomy in rats is responsible for a depression in the mobilization of plasma calcium after injection of parathyroid extract.

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THE RELATION OF PLASMA POTASSIUM LEVEL TO METABOLIC ACTIVITY

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Received for publication January 6, 1940

Sudden changes in cellular activity induced by inhibiting or exciting stimuli seem frequently to be accompanied by marked permeability changes particularly with respect to the potassium ion. This is reflected, temporarily at least, in decreases or increases in the plasma potassium.

Anesthetics and narcotics uniformly lower plasma potassium but this decrease may be prevented or converted to an actual increase by simultaneous administration of dinitrophenol (1) which has an exciting effect on general cellular metabolism.

Muscular activity is known to deplete the muscle of potassium (2) and to bring about an increase in plasma potassium (3).

Various other conditions such as severe hemorrhage (4), surgical shock (5), asphyxia (6) and tetany (7) act similarly to effect an increased plasma potassium level. In these conditions the major increase in blood potassium may be due to release of potassium from the liver, either indirectly by way of influence exerted by the secretion of the adrenals or directly by liver stimulation (8). In certain cases as in tetany, the muscular activity alone may account for the greater part of the increase. It is also conceivable that cell groups other than those mentioned may, under certain conditions, undergo changes which are reflected in the level of blood potassium.

In the following series of experiments several conditions have been devised whereby metabolic activity may be altered and the subsequent changes in serum potassium level followed.

METHODS. Food was withheld from all animals for 18 hours previous to an experiment. The dogs used were a group of normal healthy, young, adult females kept for a period of several months under the customary laboratory regime. The demedullated animals of this group were prepared some months in advance and at no time showed any sign of cortical insufficiency. The cats, as well as could be determined, were in good condition.

Potassium determinations were made by the method of Kramer and Tisdall (9). Blood sugar was determined by the method of Shaffer and

Somogyi (10) on protein free filtrates obtained by precipitation with zinc hydroxide (11). Oxygen consumption measurements were made by connecting the animal by means of a rubber mask to a Krogh spirometer equipped with a motor blower.

EXPERIMENTAL. Since the accelerating effect of cold on metabolic rate is well established we have compared the responses of normal and demedullated dogs with respect to serum potassium and blood glucose following sudden reduction of environmental temperature.

The data were obtained on two groups of three dogs each; the first group comprised normal dogs, and the second group demedullated dogs.

The lowered environmental temperature was obtained by immersing the animals in a water bath at 0°C for a period of 2 to 3 minutes. Blood

TABLE 1*

Effect of sudden cooling on the blood sugar and serum potassium of normal dogs and dogs with demedullated adrenal glands

ANIMAL NUMBER	BLOOD SUGAR		SERUM POTASSIUM	
	Before cooling	After cooling	Before cooling	After cooling
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
Normal				
1	59.5	79.8	18.3	23.6
2	72.6	89.8	17.0	19.3
3	59.5	102.0	17.6	19.9
Demedullated				
1	72.6	76.2	19.0	24.1
2	60.7	64.3	17.0	20.2
3	69.0	66.7	18.0	22.4

* These data collected in conjunction with P. S. Larson of the Medical College of Virginia.

for analysis was withdrawn from an external jugular vein immediately before immersion of the animal and again within 2 to 3 minutes following its removal from the bath.

The blood sugar and serum potassium changes induced by the experimental conditions are shown in table 1. Metabolic rate as indicated by oxygen consumption was approximately doubled for all animals during the first 10 to 15 minutes following the brief immersion in the ice bath.

It is doubtful that blood concentration could have contributed to the rise in serum potassium since the demedullated animals showed no increase in blood glucose.

These experiments indicate that under the stimulus of cold mobilization of potassium may occur without the medium of adrenal secretion. How-

ever the shivering induced might lead to the interpretation that the release of potassium is from muscle tissue alone.

To examine the mechanisms involved more exactly use has been made of the drug 2, 4 dinitrophenol recognized to be a general metabolic stimulant. It has been shown that although the metabolic activity of this drug may be depressed or nullified by certain barbiturates (12) appropriate doses may be employed so that increases in serum potassium may be effected even in the animal completely anesthetized with sodium pentobarbital (1). However such animals commonly exhibit very appreciably increased activity of the respiratory muscles. To study the rôle played by this factor serum potassium was determined following curarization.

A series of six cats were given 30 mgm. per kilo of 2, 4 dinitrophenol followed in about 30 minutes by 32 mgm. per kilo of sodium pentobarbital.

TABLE 2

The effect of dinitrophenol upon body temperature and serum potassium in the anesthetized curarized cat

ANIMAL NUMBER	CONTROL 45 MINUTES AFTER NEMBUTAL		15 MINUTES AFTER CURARE		30 MINUTES AFTER CURARE		45 MINUTES AFTER CURARE	
	T	K	T	K	T	K	T	K
	°C.	mgm. per cent	°C.	mgm. per cent	°C.	mgm. per cent	°C.	mgm. per cent
1	42.0	24.2	42.0	30.5	42.0	33.7		
2	42.0	23.4	42.5	31.7				
3	41.5	22.4			42.5	32.9	43.5	37.9
4	41.0	29.8	42.0	32.3	43.0	37.6	43.5	45.2
5	42.0	28.3	42.5	33.4	42.5	40.8		
6	41.0	22.5	42.0	34.1	42.5	39.7		

Forty-five minutes later the animals were curarized, artificial respiration started and blood samples taken at varying intervals thereafter for potassium determination. The results are incorporated in table 2.

It may be readily seen that elimination of muscular activity did not prevent the progressive rise in plasma potassium seen with the gradual increase in body temperature as the metabolic effect of the dinitrophenol progressed. The nembutal and curare may be eliminated as having any increasing effect on plasma potassium as shown by a typical protocol of a control experiment:

Cat anesthetized with nembutal. Forty-five minutes later serum K = 16.7 mgm per cent. Animal curarized. Fifteen minutes later K = 13.2 mgm. per cent, 30 minutes later K = 13.3 mgm. per cent and 45 minutes later K = 11.4 mgm. per cent.

To examine the rôle of the liver in the increase in plasma potassium occurring in response to dinitrophenol 6 cats were given dinitrophenol and

anesthetized with nembutal as before, then hepatectomized and serum potassium determinations made at intervals. These results are included in table 3 and seem to indicate that even in the absence of the liver dinitrophenol elevates blood potassium.

To eliminate simultaneously both muscular activity and liver as sources of the increased plasma potassium observed after dinitrophenol four additional cats were subjected both to hepatectomy and curarization. These

TABLE 3

The effect of dinitrophenol upon body temperature and serum potassium after hepatectomy in the anesthetized cat

ANIMAL NUMBER	CONTROL 30 MINUTES AFTER NEMBUTAL		15 MINUTES AFTER HEPATECTOMY		30 MINUTES AFTER HEPATECTOMY		45 MINUTES AFTER HEPATECTOMY	
	T	K	T	K	T	K	T	K
	°C.	mgm. per cent	°C.	mgm. per cent	°C.	mgm. per cent	°C.	mgm. per cent
1	40.0	22.0	41.0	27.4	42.0	25.1	42.0	27.9
2	39.0		40.0	21.8	40.5	28.7		
3	39.0		40.5	23.1	41.5	26.3	42.0	31.0
4	39.0	21.6			41.5	25.2		
5			40.0	21.7				
6					41.0	24.6		

TABLE 4

The effect of dinitrophenol in the anesthetized cat after hepatectomy and curarization

ANIMAL NUMBER	CONTROL 30 MINUTES AFTER HEPATECTOMY		15 MINUTES AFTER CURARIZATION		30 MINUTES AFTER CURARIZATION		45 MINUTES AFTER CURARIZATION	
	T	K	T	K	T	K	T	K
	°C.	mgm. per cent	°C.	mgm. per cent	°C.	mgm. per cent	°C.	mgm. per cent
1	41.0	24.6	41.5	31.1				
2	39.0	21.7	41.0	27.3	41.5	31.3	41.5	37.4
3	41.5	25.2	42.0	31.2	42.5	40.0		
4	40.0	21.8	40.5	28.7				

results are described in table 4 from the data of which it seems justifiable to suggest that in the absence of the liver and in the absence of muscle contractions, release of potassium from other cells in response to dinitrophenol may contribute to the total elevation observed in the plasma potassium level.

That the persisting high blood potassium values found even after curarization and hepatectomy could be due to accumulated potassium retained in the blood stream seems negated by the extreme rapidity with which injected potassium leaves the blood stream (13).

Several control experiments showed conclusively that the manipulations incidental to liver removal have no characteristic effect upon the level of plasma potassium.

Since these studies seem to indicate a close positive relationship between plasma potassium level and general cellular metabolism and one of its manifestations, body temperature, a series of cats was decerebrated, the effect of the ether allowed to dissipate and the temperature of the animal allowed to fall spontaneously or hastened in its fall by artificial cooling. The relationship between depressed metabolism and plasma potassium has been thus determined in 6 cats. The data are shown in table 5.

In several other experiments dinitrophenol was administered before, at the time of, and at varying periods after, decerebration. The results of these experiments may be summarized by saying that if the dinitrophenol

TABLE 5

The effect of decrease in body temperature due to decerebration on serum potassium in the cat

ANIMAL NUMBER	T=38° K	T=37° K	T=36° K	T=35° K	T=34° K	T=33° K	T=32° K	T=31° K	T=30° K	T=29° K
	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
Average of 7 animals	18.2									
1					11.8					10.4
2			14.4	12.9			10.4			
3				13.9	12.2					
4					14.3				10.2	
5			14.6		13.3					
6		15.8				10.9		10.1		

increased body temperature or prevented its fall in spite of the decerebration blood potassium was increased or unchanged and if body temperature first fell and was subsequently increased by the action of the dinitrophenol then blood potassium also declined primarily then rose concomitantly with the increase in body temperature.

DISCUSSION. It would seem from these experiments that there is a positive correlation between plasma potassium level and metabolic activity. That not only carbohydrate metabolism is involved (14) would seem to be indicated by the experiments upon demedullated animals in which cold brings about an increase in plasma potassium without altering blood sugar level.

Depressant drugs, known to decrease metabolic activity, and decerebration, which renders the animal unable to maintain normal body temperature, are both associated with depression of plasma potassium presumably

by its accumulation in the cells. On the other hand, excitants of metabolic activity, whether reduced environmental temperature of the normal or demedullated animal or dinitrophenol in the unanesthetized or anesthetized animal, bring about an elevation of blood potassium. Release of potassium due to muscular activity or from the liver alone or in conjunction may not be sufficient to account for the increase in blood potassium observed under the stimulus of dinitrophenol as shown by the hepatectomized, curarized animal.

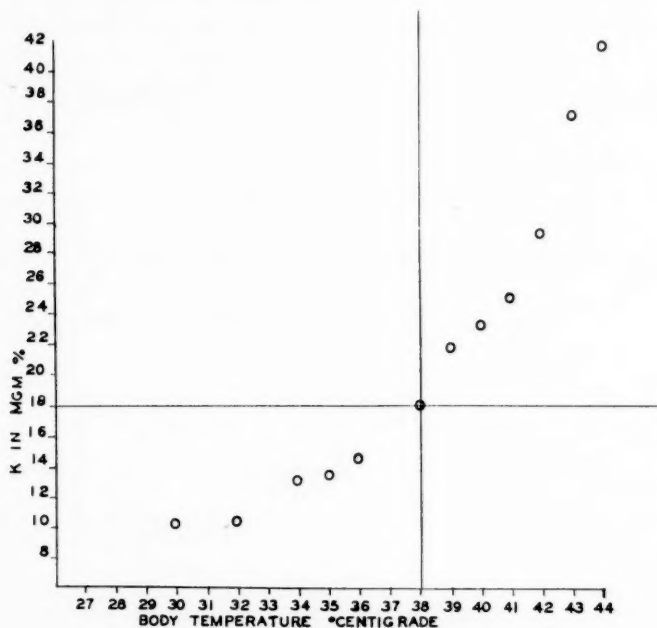


Fig. 1. Composite curve of 50 potassium determinations made in cats with body temperatures varying from 30°C. to 44°C.

Figure 1 shows a composite curve of 50 potassium determinations made in cats with body temperatures varying from 30 to 44°C. It would seem from this that within a certain range there is a direct relationship of blood potassium to body temperature. This relationship breaks down, however, at both extremes of temperature investigated; it seems probable that the sluggish circulation at low temperature and the approach of death from heat rigor at the upper temperatures could account for this. In addition under certain conditions the increase in blood potassium may far exceed

the level ordinarily associated with any given level of body temperature as shown in the following protocol:

Cat decerebrated, $T = 34.0$, $K = 13.3$ mgm. per cent.
Given dinitrophenol, one hour later $T = 37.5$, $K = 32.8$.

The 3.5° rise in body temperature in this case is associated with the same magnitude of increase as is observed in the increase in body temperature from normal to 41.5 so that the gradient of metabolic change rather than its absolute level may well be the influencing factor.

CONCLUSIONS

1. The stimulus of low environmental temperature increases plasma potassium in both normal and demedullated animals.
2. Dinitrophenol in appropriate dosage increases metabolism, body temperature and plasma potassium in anesthetized animals and this increase is not prevented by curarization, hepatectomy or both together.
3. Depression of body temperature following decerebration is associated with a depression in the plasma potassium level.
4. Decrease of body temperature and plasma potassium caused by decerebration may be nullified by dinitrophenol.
5. There would seem to be rather good positive correlation between metabolic activity and plasma potassium or more probably between change in metabolic activity and the potassium level.

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THE RENAL EXCRETION OF SUCROSE IN NORMAL MAN; COMPARISON WITH INULIN

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Received for publication January 9, 1940

Shannon (1) demonstrated in the dog as well as in man (reported by Smith (2)), that the elevation of the plasma glucose to the level of frank glycosuria completely and reversibly blocks the reabsorption of xylose and that therefore the clearance of xylose is elevated to the level of glomerular filtration. As the sucrose and xylose clearances are identical in the dogfish (3) and in the dog (4, 5, 6) and nearly identical in man (7, 8), indicating that sucrose and xylose are reabsorbed to about the same extent, it could be supposed that the elevation of the plasma glucose to the level of frank glycosuria would completely block the reabsorption of sucrose in man. Experiments directed to confirm this presumption gave the result that the sucrose clearance is not influenced by the occupation of the tubular resorptive mechanism with glucose. Moreover, contrary to the prevailing opinion, sucrose and inulin clearance seem to be identical in man, so that the glomerular filtration can also be determined by sucrose.

METHODS. Observations have been made on normal male subjects. They were examined in the morning in the basal fasting condition after having been well hydrated during the previous day. Inulin, sucrose and glucose were given by constant intravenous infusion. All the details of technic were the same as those reported in another paper (9).

Inulin and sucrose were determined by the colorimetric method of Steinitz (10), except in experiment 1, where sucrose was determined by the difference of reduction before and after hydrolysis. This proved to be inconvenient and rather difficult, as the analysis of two foreign sugars in the presence of different concentrations of glucose made the determination of 4 different control values necessary: 1, the non-glucose reducing substances of plasma; 2, the reducing substances of washed yeast; 3, the difference of reduction of the fasting plasma before and after hydrolysis, and 4, the difference of reduction of yeast before and after hydrolysis.

The colorimetric determination of the levulose component of sucrose, however, proved to be very simple and specific. Two cubic centimeters of plasma, centrifuged immediately after taking, were precipitated by the

method of Somogyi (11). The levulose color reaction was performed in two samples of the same plasma filtrate or the appropriately diluted urine, one of which had been subjected to yeast fermentation under conditions which insure complete removal of sucrose (12). The sample which had not been subjected to fermentation gave the values for the sucrose + inulin, the sample treated with yeast indicated the inulin, both expressed as levulose. The blank value of washed yeast has always been zero. For comparison a Leitz photometer (Leifo) was used. The suitability of this method for sucrose determinations has been tested on pure sucrose solutions.

In two experiments the endogenous creatinine was determined as described elsewhere (9).

TABLE 1
Osteomyelitis, S. Y., 20 years, 54.5 kgm., 160 cm., November 8, 1939

PERIOD	TIME	URINE FLOW	PLASMA LEVEL			CLEARANCE		CLEAR- ANCE RATIO	GLUCOSE REAB- SORBED
			Inulin	Sucrose	Glucose	Inulin	Sucrose		
	min.	cc. per min.	mgm. per cent	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	S/I	mgm. per min.
	0	Infusion 5 per cent inulin, 20 per cent sucrose at 10 cc. per minute							
	6	Infusion 1 per cent inulin, 4 per cent sucrose at 4 to 5 cc. per minute							
1	24-38	3.14	25.0	113.3	95	153	140.5	0.92	
2	38-54	3.35	25.0	113.3	100	110	104.5	0.95	
	59	Infusion 1 per cent inulin, 4 per cent sucrose, 20 per cent glucose at 4 to 5 cc. per minute							
3	54-95	5.02	23.5	119		147	154	1.05	
4	95-101	7.92	23.0	116.5	233	177.5	166.5	0.94	264
5	101-110	7.52	22.5	109	270	165	174	1.05	390
6	110-114	7.25	22.5	109	270	169.5	170	1.00	388

RESULTS. An experiment to demonstrate the influence of loading the glucose resorptive mechanism to capacity on the sucrose clearance is given in table 1. After having established a nearly steady level of inulin and sucrose in the plasma by constant intravenous infusion, the inulin and sucrose clearances were determined in two periods. Then 20 per cent glucose was added to the infusion solution. At the end of the 3rd period glycosuria began. During the 4th, 5th and 6th periods frank glycosuria existed and the glucose resorptive mechanism was occupied. In this patient the sucrose/inulin clearance ratio seems to be little if any affected by the existence of glycosuria. The difference of clearance ratios before (periods 1 to 3) and during glycosuria (periods 4 to 6) is too small to be interpreted as an augmentation. This first observation gave the impression that sucrose and inulin are concentrated at almost the same rate.

In table 2 the same experiment is repeated, including the endogenous

creatinine clearance. Here also no effect of glycosuria on sucrose excretion can be observed. To exclude a possible effect of inulin on the sucrose clearance, another experiment was made (table 3) in which in periods 1 to

TABLE 2

Normal subject, H., 23 years, 70 kgm., 176 cm., November 17, 1939

PERIOD	TIME	URINE FLOW	PLASMA LEVEL					CLEARANCE			CLEARANCE RATIO			GLUCOSE REABSORBED
			Inulin	Su-crose*	Creatinine	Glucose	Inulin	Su-crose	Creatinine	S/I	Cr/I	S/Cr		
			cc. per min.	mgm. per cent	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	cc. per min.					
	0	Infusion 5 per cent inulin, 20 per cent sucrose at 7.5 cc. per minute												
	8	Infusion 1 per cent inulin, 4 per cent sucrose at 4.5 cc. per minute												
1	30-37	11.0	21.1	38.0	0.64	90	169	171	183	1.01	1.09	0.93		
2	37-45	10.4	21.1	44.6			163	164	177	1.00	1.09	0.93		
	46	Infusion 1 per cent inulin, 4 per cent sucrose, 25 per cent glucose at 4.5 cc. per minute												
3	45-75	8.3	21.5	48.6			174	152	177	0.87	1.01	0.86		
4	75-85	8.5	21.6	47.0		232	190	202	194	1.06	1.02	1.04	443	
5	85-94	10.5	21.6	47.0		237	185	190	195	1.02	1.05	0.97	455	
6	94-102	11.7	21.6	47.0		252	182	175	190	0.96	1.04	0.92	413	

* Calculated as levulose.

TABLE 3

Normal subject, R. T., 25 years, 54 kgm., 164 cm., November 22, 1939

PERIOD	TIME	URINE FLOW	PLASMA LEVEL			CLEARANCE			CLEARANCE RATIO		
			Inulin	Su-crose*	Creatinine	Inulin	Su-crose	Creatinine	S/I	Cr/I	S/Cr
			cc. per min.	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	cc. per min.			
	0		Infusion 20 per cent sucrose at 10 cc. per minute								
	6		Infusion 4 per cent sucrose at 4.5 cc. per minute								
1	27-43	2.94		46.4	0.50		132	147			0.90
2	43-54	5.57		46.4	0.52		150	141.5			1.06
3	54-64	6.85		46.4	0.52		152	157.5			0.965
	65		Infusion 4 per cent sucrose, 5 per cent inulin at 12 cc. per minute								
	70		Infusion 4 per cent sucrose, 1 per cent inulin at 4.7 cc. per minute								
4	91-106	3.6	25.5	63.6	0.50	134.5	121	126	0.90	0.94	0.96
5	106-116	4.3	26.2	65.5	0.50	157.5	153	150	0.97	0.95	1.02
6	116-126	4.1	26.9	67.9	0.50	141.5	136	143.5	0.96	1.01	0.95

* Calculated as levulose.

3 only sucrose and endogenous creatinine, in periods 4 to 6 inulin, sucrose and endogenous creatinine were determined. The sucrose/creatinine ratio remained unaltered and the sucrose/inulin ratio values remained within the same range.

Table 4 represents an experiment with successively higher plasma sucrose concentrations. During the first period the plasma sucrose was maintained at the lowest concentration, at which an exact estimation can be made. In the following periods the plasma sucrose is raised successively. This experiment also gives the impression that the clearances of sucrose and inulin are identical within the limit of error and independent of the plasma sucrose level. As an average of 19 periods the sucrose/inulin ratio is 0.99.

Another series of excretion tests was made to find out whether sucrose and inulin are excreted at the same rate. According to our ideas of the excretion of inulin, the filtered quantity passes down the tubuli suffering

TABLE 4

Normal subject, A. B., 16 years, 53 kgm., 160 cm., December 5, 1939

PERIOD	TIME	URINE FLOW	PLASMA LEVEL		CLEARANCE		CLEARANCE RATIO S/I
			Inulin	Sucrose*	Inulin	Sucrose	
	min.	cc. per min.	mgm. per cent	mgm. per cent	cc. per min.	cc. per min.	
	0	Infusion 5 per cent inulin, 2.5 per cent sucrose at 10 cc. per minute					
	6	Infusion 1 per cent inulin, 0.5 per cent sucrose at 4.7 cc. per minute					
1	26-37	2.59	26.4	8.4	157	172	1.09
	40	Infusion 1 per cent inulin, 2 per cent sucrose at 4.7 cc. per minute					
2	50-62	3.21	27.2	15.6	145	145	1.00
3	62-75	3.76	26.4	22.5	149	149	1.00
	77	Infusion 1 per cent inulin, 8 per cent sucrose at 4.7 cc. per minute					
4	89-99	4.7	26.3	34.2	150	140	0.935

* Calculated as levulose.

neither increase nor decrease. If it be assumed that the excretion of another substance is subject to the same laws, this substance ought to be excreted at the same percentage of the injected quantity as inulin, if it is injected simultaneously. In fasting subjects exactly 40 cc. of 5 per cent inulin and 10 per cent sucrose in 0.85 per cent saline were injected intravenously after previous voiding of the bladder. A sample of this solution was diluted and analyzed for its content of inulin and sucrose. In six subjects examined (table 5) the excretion of inulin and sucrose was identical during the first hour. These results make it rather improbable that any tubular reabsorption is involved in the process of sucrose excretion.

The data of the excretion ratio of the second and third hour, however,

TABLE 5

SUBJECT	TIME	URINE	INULIN INJECTED	SUCROSE INJECTED	EXCRETION PERCENT- AGE OF INJECTED		EXCRETION RATIO S, I
			Calculated as levulose		Inulin	Sucrose	
			grams	grams			
I. A.	0		1.86	1.96			
	60	61			61.3	60.1	0.98
	120	54			14.0	18.8	1.34
	180	52			8.5	12.5	1.47
Total.....					83.8	91.4	1.10
N. B.	0		1.86	1.96			
	60	48			49.0	50.7	1.03
	120	31			17.3	21.3	1.23
	180	25			8.9	13.0	1.46
Total.....					75.2	84.0	1.12
S. S.	0		1.86	1.96			
	60	68			39.3	38.4	0.98
	120	52			15.2	16.4	1.08
	180	84			11.6	12.3	1.06
Total.....					66.1	67.1	1.02
N.	0		1.76	1.80			
	60	58			57.8	58.8	1.02
	120	55			17.5	23.8	1.36
	180	60			10.8	12.0	1.11
Total.....					86.1	94.6	1.10
L.	0		1.76	1.80			
	60	43			65.3	65.0	1.00
	120	18			15.4	24.0	1.56
	180	17			5.6	9.3	1.66
Total.....					86.3	98.3	1.14
A.	0		1.76	1.80			
	60	285			67.6	66.2	0.98
	120	18			3.8	5.6	1.47
	180	30			6.2	8.3	1.34
Total.....					77.6	80.1	1.03

are difficult to interpret. In all cases, except one, sucrose was excreted at a distinctly higher percentage than inulin. Without further investigation an explanation for this behavior cannot be given. After the in-

travenous administration of 2 grams sucrose (calculated as levulose) the average plasma value during the second hour is about 5 mgm. per cent, the average plasma value during the third hour about 2.5 mgm. per cent. Neither inulin nor sucrose could be determined with sufficient accuracy at these low plasma levels, so that no exact clearance determinations could be made.

DISCUSSION. A direct comparison of sucrose and inulin clearance has not yet been made. Smith and his collaborators (4, 5, 6) found that in the dog sucrose and xylose are reabsorbed to about the same extent. In man, two series of observations on this subject have been made. Keith, Power and Petersen (7) found that the sucrose clearance is generally about 10 per cent higher than the xylose clearance. Smith (8) reports observations of Chasis in which the sucrose/xylose ratio is constantly higher than 1.0, on the average 1.08. "The fact that in these experiments the sucrose/xylose ratio is greater than 1 is not evidence, in the author's opinion, that these sugars are handled differently, as White and Monaghan (13) have argued. Two milligrams of non filtrable reducing substance in plasma would reduce the discrepancies of these sugar clearances below the significant difference (± 5 per cent)." Although xylose has not been compared with sucrose in our observations and therefore no further evidence to this question can be given, the observations of these two authors seem to indicate that sucrose and xylose are not treated in the same manner in man.

Winkler and Parra (14) stated that the exogenous creatinine clearance is always higher than the sucrose clearance. The plasma concentrations of creatinine used by these authors lie between 25 and 8 mgm. per cent. In these concentrations the creatinine/inulin ratio is known to be 1.40 (Shannon, 15). If the creatinine/sucrose ratio is calculated from the 28 periods of Winkler and Parra, the average value of 1.36 is found. The exogenous creatinine clearance is elevated above the inulin clearance as much as above the sucrose clearance. This fact seems to provide further evidence that sucrose is excreted without tubular reabsorption.

The evaluation of the sucrose clearance in dogs is complicated by another fact. Contrary to man enzymes capable of inverting sucrose could be found in the blood and in the urine of dogs (Keith and Power, 16; Abderhalden and Buadze, 17). In man such invertases have not been detected (16, 18). Although these invertases are rarely to be found before a certain delay of time, Keith and Power were never able to find a quantitative excretion of sucrose in dogs. Perhaps these facts can explain the discrepancy between the results of Jolliffe, Shannon and Smith (4, 5) and of Pitts (6) at one hand and of White and Monaghan (13) at the other hand.

The fact that after simultaneous injection of sucrose and inulin the

excretion ratio during the first hour is 1.0, while during the second and third hour sucrose is concentrated more than inulin, cannot be explained without further investigation. A tubular secretion of sucrose at very low plasma levels is as improbable as a tubular reabsorption of inulin at very low plasma levels, but in the absence of an adequate method to determine these minimal quantities in the plasma no decisive conclusion can be advanced on this subject.

SUMMARY

The sucrose/inulin clearance ratio in normal man is not influenced by the occupation of the tubular resorptive mechanism with glucose.

The simultaneous clearances of sucrose and inulin are practically identical. The average value of the sucrose/inulin ratio of 19 periods is 0.99.

The author wishes to thank Mrs. S. Hersch for collaboration.

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EFFECTS OF ADRENALIN ON TUBAL CONTRACTIONS OF THE
RABBIT IN RELATION TO THE SEX HORMONES
(STUDY IN VIVO BY RUBIN METHOD)

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Received for publication January 9, 1940

It has been shown that during estrus or after treatment with estrogens, there is an increase in amplitude and number of spontaneous contractions of the uterus (Frank, Bonham and Gustavson, 1935; Reynolds, 1931; Robson, 1933). It is also known that the gravid and non-gravid uterus of the rabbit contracts when acted upon by adrenalin (Langley, 1901; Cow, 1918; Sauer, Jett-Jackson and Reynolds, 1935). Few studies, however, have been made on the pharmacodynamics of the fallopian tubes, especially in vivo. Morse and Rubin (1937) first employed the Rubin method to demonstrate pharmacodynamic effects on the tubal contractions of the monkey's oviducts. In the present communication, the effect of physiologic doses of intravenous adrenalin upon the intact oviducts of the rabbit during different hormonal states are reported. The dynamics of the uterine salpinx were recorded by the kymographic technique of tubal insufflation as employed by Rubin in the clinical test for tubal patency since 1925. This method accurately records tubal contractions in vivo as well as in the surviving extirpated organs.

The observations on the rabbit's oviducts were made during anestrus, estrus, after castration, and following the administration of estrogenic and androgenic substances.

MATERIAL AND METHODS. Rabbits anesthetized with nembutal were used in all experiments. The tubal contractions were recorded by the Rubin tubal insufflation kymographic apparatus. The oviducts were exposed through a laparotomy. A small incision was made in the uterine horn just proximal to the utero-tubal junction. Through this opening a glass cannula was inserted into the uterine cavity and securely tied. The oviduct was then insufflated with CO₂ and the contractions were thus recorded. The CO₂ was delivered under a constant pressure of fifteen pounds and constant-flow rate of 1 cc. per second. The same rate of flow

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² Aided by grants from Josiah Macy, Jr. Foundation and Drazian Fund for Medical Research.

was used in all experiments. Wimpfheimer and Feresten (1939a) showed by this technique that the manometric fluctuations were due solely to changes in tone of tubal musculature. Furthermore, in several experi-

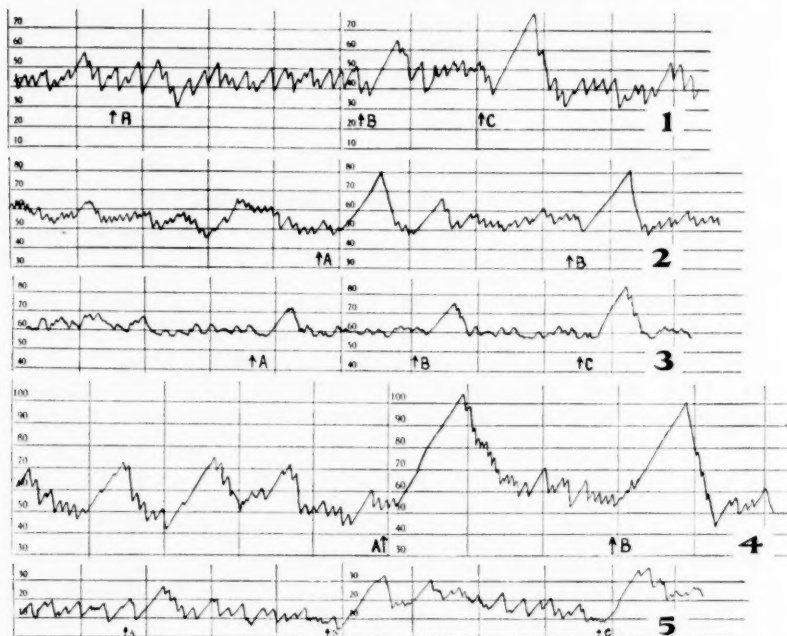


Fig. 1. Tubal contractions of a rabbit in anestrus, weight 3.2 kgm. Adrenalin 1.0, 3.0 and 4.0 gammas injected at points A, B and C respectively. Ordinate, millimeters of mercury; abscissa, time in minutes.

Fig. 2. Tubal contractions of a rabbit in estrus. Adrenalin 0.2 gamma injected at points A and B. Ordinate and abscissa, same as in figure 1.

Fig. 3. Tubal contractions of a castrated rabbit weight 3.3 kgm. Adrenalin 2.0, 3.0 and 4.0 gamma injected at points A, B and C respectively. Ordinate and abscissa are the same.

Fig. 4. Tubal contractions of a rabbit following injection of 2000 International Units of Estradiol Benzoate. Weight 3.6 kgm. Adrenalin 0.1 gamma injected at points A and B.

Fig. 5. Tubal contractions of a rabbit after administration of 200 mgm. of Testosterone Propionate. Weight 3.0 kgm. Adrenalin 10, 50 and 100 gamma injected at A, B and C respectively. Note the low mean pressure level as compared with the above curves.

ments the tube was severed at or distal to the utero-tubal junction and insufflated through its fimbriated end thus eliminating uterine activity. The results obtained did not differ from those in which the oviducts were insufflated from the uterine end as described above.

Varying concentrations of epinephrine were injected into the marginal veins of the ear. A constant volume (1.0 cc.) and constant speed of injection were used in all trials. The dilutions of epinephrine were made with normal saline. Some of the rabbits were tested during anestrus and the resultant tubal responses were later compared in the same rabbit with those obtained during estrus or following the injection of estrogenic or androgenic hormones.

RESULTS. a. *Anestrus.* The oviducts of anestrus rabbits responded with varying amounts of epinephrine. Sustained contractions of 20 to 40 mm. Hg in the oviduct, lasting from 30 to 40 seconds, were obtained with minimal doses of between 2.0 and 8.0 gammas per rabbit. The average minimal dose necessary to produce a sustained contraction of 20 to 40 mm. of mercury was 3.6 gammas per rabbit.

b. *Estrus.* When these rabbits were tested during estrus, it was found that the oviducts became extremely sensitive to epinephrine. During estrus a sustained contraction of the tube could be produced with threshold doses varying from 0.1 to 0.5 gamma of epinephrine. The average dose required to produce a sustained contraction of from 20 to 40 mm. of mercury in the tube of the estrus rabbit was 0.2 gamma of epinephrine per rabbit. In other words, estrus sensitized the tubal response to adrenalin more than tenfold.

c. *After castration.* The rabbits were surgically castrated and were insufflated three weeks later. Minimal doses of 4.0 to 5.0 gammas of epinephrine were necessary to elicit sustained contractions of 20 to 40 mm. of mercury. It appeared that castration did not appreciably alter the response of the oviduct from that of the anestrus rabbit.

d. *Estradiol benzoate.* Anestrus and castrate rabbits were injected intramuscularly with divided doses of estradiol benzoate, the total doses varying from 2000 to 4000 international units. The oviducts were insufflated two days after the last injection, and strong contractions of 20 to 40 mm. Hg were obtained with amounts as small as 0.04 gamma of epinephrine. Estrogens increased the sensitivity of tubal response to adrenalin even more conspicuously than did physiologic estrus.

e. *Testosterone propionate.* Estrus and anestrus rabbits were injected with testosterone propionate over a two-week period. Total doses varying from 100.0 to 200.0 mgm. were used. The minimal dose for eliciting a tubal contraction was markedly increased. Responses of 20 to 40 mm. Hg were only obtained after amounts as high as 10.0 to 50.0 gammas of epinephrine. Evidently androgens markedly decreased the tubal response to adrenaline.

DISCUSSION. The spontaneous contractions of the oviducts are governed by the hormonal status (Corner, 1923; Wislocki and Guttmacher, 1924; Sackinger and Snyder, 1926). With castration there is a marked reduction in the frequency and amplitude of tubal contractions and but

slight decrease in the mean pressure level. It is assumed that this level is proportional to the tonicity of the tubal musculature. These normal contractions may be restored after the administration of estrogens. Estrogens produce an increase in number and amplitude of contractions, which are maintained at higher mean pressure levels (Wimpfheimer and Feresten, 1939b). Following the injection of androgens, the tubal contractions, although not decreased in number, are of low amplitude and are maintained at low mean pressure levels. It is to be noted that the mean pressure levels at which tubal contractions are maintained after androgens is much lower than after castration (Rubin and Davids, 1940).

The foregoing experiments also show that the tubal musculature of the rabbit contracts in response to epinephrine injected into the circulation. The sensitivity of the oviducts to adrenaline is conspicuously altered by the hormonal status. During estrus, contractions in the tube can be obtained with doses much smaller than those necessary to produce the same degree of contraction in the anestrus stage. Similar hypersensitivity to adrenaline may be obtained by injecting exogenous estrogenic substances. When, however, an androgen is administered to the female rabbit, the sensitivity of the oviduct to epinephrine is greatly lowered. There are no noticeable alterations in the epinephrine response of the tube after castration, the dose necessary to elicit an arbitrary contraction being similar to that in the anestrus stage.

From these studies, there appears to be an interrelationship between certain sex hormones and at least one autonomic hormone, namely epinephrine. An increase in concentration of estrogenic hormone, whether it be endogenous or exogenous, enhances the effect of epinephrine on the oviduct of the rabbit, while androgens depress the epinephrine effect.

Whether this relationship depends on the structural changes produced by the sex hormone, such as hypertrophy of the tubal musculature observed after injecting estrogens (Wimpfheimer and Feresten, 1939b) or on some qualitative alterations in muscle tone, or on both, is difficult to determine. It is probable that the type of epinephrine contraction is dependent on the initial tone in the fallopian tube tested. As far as these experiments go, they suggest that the action of the autonomic adrenergic hormone on the genital organs is affected by sex hormones secreted in the body. This principle of interaction between endocrine secretions is analogous to the increased action of adrenalin on the heart in the presence of increased thyroid hormone (Wise and Hoff, 1938).

SUMMARY

1. Adrenalin in small amounts injected intravenously in the anestrus rabbit produces a definite increase in the contractility of the intact oviducts.

2. The tubal response to epinephrine is increased during estrus or after injection of estrogens and decreased after the injection of androgens.

3. Castration produces no significant change in the adrenalin response of the oviduct.

We are indebted to Dr. I. C. Rubin for his valuable suggestions and aid in these experiments.

We wish to thank Roche Organon Company for the Testosterone Propionate (Neo-Hombreol) and Estradiol Benzoate (Dimenformon Benzoate) used in these experiments.

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THE EFFECT OF CHANGES IN MUSCLE ELECTROLYTE ON THE RESPONSE OF SKELETAL MUSCLE TO TETANIC STIMULATION WITH PARTICULAR REFERENCE TO POTASSIUM¹

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Received for publication January 10, 1940

Previous work has demonstrated profound effects of potassium on the contraction of voluntary muscle. The work in general has consisted in determining the contractility of striated muscle immersed in or perfused by solutions containing varying concentrations of potassium. Careful studies by Hegnauer, Fenn and Cobb (1) and Fenn and Cobb (2) showed that surviving voluntary muscle of frogs immersed in ordinary Ringer's solution, which contains 2 milliequivalents of potassium per liter of solution, quickly lost its ability to contract and at the same time the potassium in the muscle cells and the oxygen consumed by them were found to decrease. They found that increasing the concentration of potassium in the Ringer's solution augmented the contraction of the muscle, reaching optimum at a concentration of between 5 and 6 milliequivalents, but that further increases in concentration of potassium were accompanied by a loss of contractility. Increasing the concentration of potassium beyond 7.7 milliequivalents not only caused a decrease in muscle contractility but also an increase in oxygen consumption and an increase in the amount of potassium entering the muscle cell. Perfusing the legs of cats, Baetjer (3) found that the administration of from 0.05 to 0.2 milliequivalent of KCl into the artery of the leg caused a temporary increase in the contraction of the muscles of the perfused leg even after curare. Brown (4) and Brown and von Euler (5) likewise perfused the legs of cats with solutions containing similar small amounts of KCl and concluded that voluntary muscle was very sensitive to changes in the potassium balance within and without the muscle fibre and that small changes in this balance might lead to either a depression or potentiation of the twitch tension of the muscle.

While this previous work has indicated that muscle activity may be affected by rapid changes in extracellular electrolyte, data are not available to show whether or not the electrolyte pattern within the muscle cell

¹ Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

affects the ability of the cell to perform work. Methods are now available for increasing and decreasing the potassium within the muscle cell without interrupting the neuromuscular connections or injecting potassium salts into the blood stream (6) (7). The alterations thus produced in muscle potassium occur over a period of days and simulate the changes in potassium as they probably occur in adrenal insufficiency and other disturbances. Using these methods for altering the potassium of skeletal muscle, we have studied the response of the muscle to repeated tetanic stimulation.

EXPERIMENTAL PROCEDURE. Healthy male albino rats weighing between 200 and 300 grams were used in all experiments. The following procedures were used to vary the potassium concentration of the serum and muscle cell: 1, total adrenalectomy; 2, sodium depletion by means of the intraperitoneal injection of 5 per cent solution of glucose; 3, a diet low in potassium, and 4, a diet low in potassium given to rats that were subsequently subjected to total adrenalectomy. The rats in groups 1 and 2 were maintained throughout on a stock diet of Purina Dog Chow, which on analysis was found to contain 0.6 per cent potassium. The rats in group 1 were allowed to survive from 76 to 216 hours after adrenalectomy but were not allowed to reach the terminal stage of adrenal insufficiency. The rats in group 2 were depleted of sodium by injecting 25 to 35 cc. of 5 per cent solution of glucose intraperitoneally and removing the fluid from the peritoneum as completely as possible after four to five hours. This procedure was carried out either once or twice in each rat. The animals were allowed to survive from five to twenty-four hours after the last fluid was removed, so that electrolyte equilibrium might be reached. The diet fed to the rats in groups 3 and 4 had the following ratio: lactalbumin 18 grams, sucrose 25 grams, dextrin 32 grams, crisco 22 grams, cod liver oil 1 gram, yeast powder 2 grams, bone ash 2 grams and sodium chloride 1 gram. This diet on analysis was found to contain 1.4 milliequivalents of potassium per 100 grams of dried weight of food. The rats were kept on this diet from 14 to 35 days before they were killed. From 7 to 10 grams of diet a day were consumed and although in some of the heavier animals there was an occasional early loss of weight, most of the rats held their weight or gained from the beginning to the end of the experiment. The rats in group 4 were allowed to survive from fifteen to twenty-six days after total adrenalectomy. A fifth group of rats served as controls and were maintained on the stock diet. No therapy was given any animal. Water and food were given *ad libitum*.

Each rat was anesthetized preliminary to the stimulation by the intraperitoneal injection of 5 to 10 mgm. of nembutal. Stimulation was carried out according to the method described by Everse and De Fremery (8). One hind foot was attached to the recording lever of a torsion wire myograph by fine wire. Through a slit in the skin of the leg a silver dipolar

electrode was inserted into the belly of the gastrocnemius soleus muscle. The current was led off from the secondary circuit of a Harvard induction coil which was set to give supramaximal make and break shocks at the rate of 60 per second. The primary circuit of the induction coil was attached to a dry cell battery. The period of stimulation in each animal was the same and is as described in figure 1. Immediately following the period of stimulation the animal was anesthetized more deeply by inhalation of ether and blood was withdrawn under oil from the abdominal aorta. The muscles of both hind legs and abdomen were dissected off, put in a covered receptacle, weighed and dried to constant weight at 100° to 105°C. The chemical analysis of the serum and muscle were carried out in the same manner as described in a previous communication (6).

RESULTS. The types of curves obtained were similar to those described by Everse and De Fremery. The decision as to whether or not a particular curve was normal was made by comparing the average height of the last three curves with the height of the initial curve. In nine out

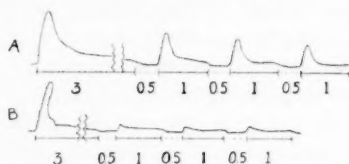


Fig. 1. Tracings of sample myographic recordings resulting from tetanic stimulation of gastrocnemius soleus muscle of rats. A, normal curve; B, abnormal curve. Solid line beneath tracing represents period of stimulation and dotted line, period of rest. Numbers represent minutes.

of ten control rats kept on the stock diet (Purina Dog Chow) the average height of the last three curves was found to be from 50 to 63 per cent of the height of the initial curve. In the tenth rat it was 37 per cent. In thirty rats in which attempts were made to change the potassium concentration of the skeletal muscle it was found that the average height of the last three curves amounted to from 0 to 63 per cent of the initial curve. In thirteen of these thirty rats the average height of the three curves was 30 per cent or less of the height of the first curve. The responses to the tetanic stimulation obtained in these thirteen rats were considered to be abnormal, because the ratio of the average height of the last three curves to the height of the initial curve was well below that found in any of the ten control animals. The rats with abnormal curves consisted of six of the seventeen rats that were totally adrenalectomized (group 1), four of the six rats depleted of sodium (group 2), and the three rats on the low potassium diet which were subsequently adrenalectomized (group 4). None of the four rats on the low potassium diet in group 3 had abnormal curves.

The type of curve obtained in each rat was compared with the electrolyte pattern of the rat's serum and muscle with particular attention directed toward the potassium of the muscle. In order to conserve space certain rats from the group of thirty experimental animals have been selected to illustrate the lack of correlation between the type of curve resulting from the tetanic stimulation and the potassium in the muscle (table 1).

TABLE 1

The type of response to tetanic stimulation observed in the intact rat according to the muscle and electrolyte pattern

GROUP	RAT NUMBER	HEIGHT* OF CURVE	PER L. SERUM ULTRAFILTRATE				MUSCLE ELECTROLYTE									
			Na	Cl	K†	NPN‡	Per 100 grams fat-free solids								Per 1000 cc. intracellular water	
							Na	Cl	K	P	Prot.	Total H ₂ O	Intracell. H ₂ O	K	Prot.	
		per cent	mM	mM	mM	mg. per cent	m.eq.	m.eq.	m.eq.	m.eq.	grams	cc.	cc.	mM	grams	
I	29	10.3	143	112	4.9	120	9.5	7.4	47.8	32.4	94.4	337	271	175	348	
	15	15.4	139	118	7.5		8.8	8.0	52.6	34.4	98.7	352	284	184	348	
	14	63.0	137	110	4.9		10.3	8.2	51.2	34.7	98.4	372	298	171	330	
II	38	0.0	127	89	5.6		6.8	5.2	49.1	32.2	90.1	381	323	152	280	
	41	10.2	122	98	7.6	130	7.4	6.3	52.2	35.7	92.8	381	316	164	294	
	42	48.8	133	99	7.8	204	8.0	6.5	52.3	35.2	93.8	374	308	169	304	
III	33	50.3	147	117	3.5	38	15.9	8.8	40.0	30.6	92.4	374	299	133	309	
IV	34	0.0	142	111	5.5	60	13.3	8.0	42.0	32.2	92.5	344	271	154	342	
	36	0.0	133		8.7	96	10.0	8.4	47.9	32.5	94.3					
Normal (seventeen rats)§			146.3 ±2.4	112.6 ±2.6	4.28 ±0.77		10.25 ±0.62	7.37 ±0.6	49.16 ±0.87	33.65 ±1.03	92.95 ±2.11	344 ±5.77	278.2 ±5.77	176.3 ±3.1	339.1 ±12.6	

Group I, Purina dog chow diet. Bilateral adrenalectomy.

Group II, Purina dog chow diet. Sodium depleted.

Group III, Low potassium diet.

Group IV, Low potassium diet. Bilateral adrenalectomy.

Normal, Purina dog chow diet.

* Refers to the ratio between the average height of the last three curves and the height of the initial curve resulting from the three-minute period of stimulation.

† Millimoles per 1000 cc. of serum.

‡ Milligrams per 100 cc. of serum.

§ The average result and the standard deviation from the mean are given.

Similar results to those given in table 1 occurred among the remaining animals and only served to confirm the findings presented in the table. Also in the table are given the results of the analysis of the serum and muscle of seventeen normal control rats maintained on Purina Dog Chow, so that the electrolyte findings in the experimental rats may be compared with those of normal control rats.

The figures in column 1 of the above table show that rats 14, 42 and 33

had normal curves, while the remaining rats had abnormal curves. Comparing the type of curve (column 1) with the potassium content of the muscle (column 8), it is observed that the normal curves in rats 14 and 42 were associated with muscle potassiums that are higher than normal. The normal curve in rat 33 was associated with a very low muscle potassium. On the other hand, abnormal curves in rats 29 and 38 were associated with normal muscle potassium and in rats 15 and 41 with high muscle potassiums and in rat 34 with a very low amount of potassium in the muscle. The high potassium content of the skeletal muscle (column 8) found in rats 15, 14, 41 and 42 are far enough above the normal range (49.16 ± 0.87) to be significant even though they are single experiments. Likewise the low muscle potassiums in rats 33 and 34 are significantly decreased below the normal range. Furthermore, no correlation could be found between the potassium concentration in the serum (column 4) or in the intracellular water (column 13) and the normality or abnormality of the curves in the individual rats.

Fenn and Cobb (10, 11) have demonstrated that tetanic stimulation of voluntary muscle leads to a loss of potassium from the muscle and an increase in extracellular water and chloride. These changes were observed after rather long periods of stimulation (30 min. or more). In order to ascertain whether the amount of stimulation used in the present experiments produced similar changes in electrolyte, the electrolyte of the pooled muscles from the stimulated legs of four normal rats was compared with that of the muscles from the non-stimulated legs of the same rats. A similar procedure was carried out on three adrenalectomized rats. The results are shown in table 2.

It is seen that there is a gain in muscle sodium, chloride and total water in the stimulated muscles of both normal and adrenalectomized muscles (columns 4, 5 and 7).

Since the table shows an increase in sodium which is relatively greater than the increase in chloride, only part of the added muscle sodium can be extracellular. Apparently some augmentation in intracellular sodium occurs in even a short period of stimulation but our data do not indicate any loss of intracellular potassium as was found by Fenn after longer periods of stimulation. In any case the results indicate that the changes in muscle electrolyte accompanying stimulation are not sufficient to invalidate our analyses as indications of the composition of muscle before stimulation.

DISCUSSION. Since the above experiments indicate that the concentration of potassium in muscle cells may vary over a wide range without altering contractility, some other factors must have been operating to produce the abnormal curves obtained in certain rats. In the present experiments, impaired circulation of considerable degree was met in all

animals with abnormal curves. The evidences of circulatory failure were marked cyanosis of the skin, dark color of the arterial blood and the fact that only 1 or 2 cc. of blood could be obtained from the abdominal aorta whereas 5 or 6 cc. could be obtained from normal animals or experimental animals with normal curves. Furthermore, typically abnormal curves could be produced in a normal control rat by simply clamping the femoral artery of the stimulated leg throughout the period of stimulation, indicating that the failure of the muscle to respond normally to subsequent tetanic stimulation occurred during the aerobic phase of muscle metabolism.

Our failure to alter muscle activity even with marked changes in muscle potassium might appear to be contradictory to the results obtained by

TABLE 2

Electrolyte in stimulated muscles compared with electrolyte in non-stimulated muscles of normal and adrenalectomized rats

	PER L. SERUM ULTRAFILTRATE			MUSCLE							
				Per 100 grams fat-free solids						Per 1000 cc. intracellular water	
	Na	Cl	K*	Na	Cl	K	Total water	Extra cell. water	Intra cell. water	K	Prot.
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	mM	mM	mM	m.eq.	m.eq.	m.eq.	cc.	cc.	cc.	mM	grams
Normal (4) stimulated...	140.5	116.6	3.43	14.8	11.4	48.2	405	98	307	156	318
Non-stimulated.....				10.5	9.3	47.8	381	80	301	159	319
Adren.† (3) stimulated...	147.7	116.0	5.2	11.8	9.7	49.9	383	83	300	166	316
Non-stimulated.....				9.5	8.1	49.2	355	70	285	172	331

* Millimoles per 1000 cc. of serum.

† Adrenalectomized.

previous investigators. It must be realized, however, that the present experiments differed from previous ones in two important respects. The profound changes in muscle potassium observed by us were produced gradually and were largely confined to changes in intracellular potassium. The perfusion experiments, on the other hand, dealt with very rapid changes in potassium and largely with changes in potassium in extracellular fluid. While rapid changes in serum potassium may produce changes in the intracellular concentration of electrolyte of muscle, our data indicate that the composition of muscle with respect to electrolyte does not of itself affect contractility.

The present data may be used in the interpretation of the effects of variations in the composition of muscle and serum with respect to sodium

and potassium as met in adrenal insufficiency, deficit of extracellular electrolyte and other physiological disturbances. In these conditions, the changes in composition are slow in appearing and not undergoing rapid alterations. Our results do not support the belief that the muscular fatigue of adrenal insufficiency is specifically related to the alteration in muscle or serum potassium. Furthermore the failure to demonstrate loss of contractility in rats with marked deficits in muscle potassium and low concentration of potassium in serum indicates that disturbances in muscle potassium are not per se the explanation of familial periodic paralysis. This phase of our work confirms Heppel and supports the views of Pudney et al. (12) that the muscular weakness is not due to the lowering of serum potassium or changes in muscle potassium.

SUMMARY

1. The serum and muscle electrolyte of adult male rats were altered, particularly as regards potassium, by various methods and the relation between composition and the response of the skeletal muscle to a direct tetanic stimulation was studied.

2. No direct relation was found between the amount of potassium in the muscle or serum and the type of response to the tetanic stimulation obtained.

3. It is felt that this lack of correlation in the data presented is evidence against the belief that the myasthenia noted in adrenal insufficiency is due specifically to increases in the potassium of skeletal muscle. *

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CHANGES IN CHOLESTEROL CONTENT OF HEPATIC BILE SUBJECTED TO GALL-BLADDER ACTIVITY¹

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Received for publication January 11, 1940

In 1931, Elman and Taussig (1) reported that in the dog cholesterol was secreted into the gall-bladder bile. Studies made in this laboratory (2, 8) suggested that the normal gall-bladder wall neither secreted nor absorbed cholesterol, although secretion of cholesterol by the damaged gall-bladder wall often occurred. In 1935, Wilkie and Doubilet (3) published data which suggested that the direction of passage of cholesterol through the normal gall-bladder wall was determined by the blood-bile cholesterol ratio and in 1936 Rousselot and Baumann (4) reported that cholesterol was absorbed from the gall-bladder bile. Since our original report more satisfactory methods of analysis have led us to repeat and extend our previous experiments.

METHODS. The dogs were prepared for the experiments in the manner described in a previous paper (5). In fourteen animals the bile was introduced into and removed from the bile-free gall bladder as previously reported (2). In two animals bile was introduced into the gall bladder through the choledochocystic duct catheter which was then withdrawn and the isolated portion of the common duct closed so that no gall-bladder bile could escape through the ductal system.

At the termination of the experiment, following removal of the bile and lavage of the gall bladder with 0.9 per cent sodium chloride, a solution of methylene blue was injected through the catheter into the gall bladder and the catheter clamped. The animal was then killed and the gall bladder carefully dissected away from the liver bed. Open accessory ducts, if present, could be detected by the escape of methylene blue from the gall-bladder lumen. A section of the gall-bladder wall was fixed for histologic study.

In some experiments a specimen of blood for cholesterol determination was taken before operation and at the end of the experiment. Blood and bile cholesterol determinations were made by precipitation with digitonin and subsequent colorimetric measurement (6).

¹ Aided by a Grant from the Josiah Macy, Jr. Foundation.

In five dogs 0.9 per cent sodium chloride solution instead of bile was introduced into the gall bladder, withdrawn immediately and the gall bladder then washed with the same solution. In three animals the sodium chloride solution was allowed to remain in the gall bladder for twenty-four to forty-eight hours, withdrawn, and then the gall bladder washed with fresh solution. The fluid so obtained was analyzed for its cholesterol content.

RESULTS. *Accessory ducts.* We wish to emphasize again the fact that in the dog accessory bile ducts are almost invariably present and frequently are so located as to make them invisible at operation. Of a total of forty-three dogs operated on for this series of experiments eighteen had to be discarded because of the presence of accessory bile ducts on the posterior surface of the gall bladder where they could not be seen and ligated at operation, while other animals could not be used because of vascular abnormalities which prevented a satisfactory preparation.

It is essential for a satisfactory solution of this problem that all accessory ducts be ligated without injury to the vascular or lymphatic systems of the gall bladder. It is possible that in some instances where other investigators have found large increases in the amount of cholesterol, the increases may have been due to bile flowing into the gall bladder from unligated accessory ducts. We have obtained such data in gall bladders in which unligated accessory ducts were present, even when the gall bladders of these animals were satisfactory for the experiment in other respects (dogs 22, 23 and 24).

Volume. In all of our satisfactory experiments the volume of bile in the gall bladder decreased markedly during the experimental period. This decrease in volume is an essential point in the experiment, since it is one factor which indicates a normally functioning gall-bladder wall. It has been shown that after the mucosa has been damaged the gall bladder loses its ability normally to concentrate the bile and indeed when serious injury of the wall has occurred fluid is secreted into its lumen. In most instances a volume of 20 cc. of mixed gall-bladder and liver bile introduced into the normal gall bladder diminished to approximately 5 cc. in the course of eight hours. We have, as a rule, placed from 10 to 20 cc. of bile (the average capacity of the gall bladder of a 10 kgm. dog) in the gall bladder and then, as concentration took place, added to the gall-bladder contents at two intervals during the twenty-four hour period. At the conclusion of an experiment we usually recovered from 7 to 9 cc. of concentrated bile for analysis.

In the two experiments where a known amount of bile was placed in the gall bladder, the catheter withdrawn and the opening in the duct ligated (dogs 9 and 12) no further addition of bile could be made. At the end of the twenty-four hour period practically all fluid had been ab-

sorbed, leaving less than 1 cc. of viscous bile in the gall bladder. This is invariably the situation when 10 to 20 cc. are placed in the gall bladder for twenty-four hours with no further additions. We are of the opinion that in any experiments of this type lasting twenty-four hours or more, if fluid in any measurable amount is recovered it is due either to damage to the gall-bladder wall or to the presence of unligated accessory ducts.

Saline controls. When the gall bladder was washed out with 3 x 10 cc. of 0.9 per cent sodium chloride, the washings contained no cholesterol, which indicated that the mucosal cells were not washed away in large numbers by the salt solution. Only a trace of cholesterol was found in the gall-bladder contents and washings twenty-four hours after the introduction of 0.9 per cent sodium chloride (table 1). This seems to

TABLE 1
Saline controls

DOG	VOLUME		CHOLESTEROL			TIME hours	MICROSCOPIC
	In	Out	In	Out	Dif.		
	cc.	cc.	mgm.	mgm.	mgm.		
1	20.0	20.0	0	0	0	0	
2	12.0	12.0	0	0	0	0	
3	12.0	12.0	0	0	0	0	
4	45.0	45.0	0	0	0	0	
5	45.0	45.0	0	Faint trace	Faint trace	0	
6	45.0	1.0	0	Faint trace	Faint trace	28	Edema +++
7*	45.0	0	0	Trace	Trace	24	Edema +++
8*	30.0	0	0	1.4	1.4	24	Mucosa completely disintegrated

* Washed with saline.

us additional evidence that the mucosa does not normally secrete cholesterol. The only experiment in which more than a trace of cholesterol was removed (dog 8) was that in which a large portion of the gall-bladder mucosa at the end of the twenty-four hour experimental period became detached.

Blood cholesterol. According to Wilkie and Doubilet (3) over a period of twenty-four hours, "when the cholesterol concentration of the bile is lower than that of the blood, cholesterol passes from the blood through the mucosa of the gall bladder into the bile; when the cholesterol concentration of the bile is higher than that of the blood, cholesterol passes from the bile through the mucosa of the gall bladder into the blood stream." Our data do not support this concept (table 2). For example, in dog 10 the cholesterol concentration in the bile introduced was considerably

less than the concentration of cholesterol in the blood, but there was a gain of only 1.1 mgm. of total cholesterol in the bile, and the concentration of cholesterol (115 mgm. per cent) in the bile at the end of twenty-seven hours was not equal to that in the blood (250 mgm. per cent). In dog 9 the cholesterol concentration in the bile at the beginning of the experiment (208 mgm. per cent) was slightly higher than the cholesterol concentration in the blood (161 mgm. per cent) which, according to Wilkie and Doubilet (3) should have resulted in a loss of cholesterol from the gall bladder. After twenty-three and one-half hours the volume of solution in the gall bladder had decreased to so small an amount that it could not be withdrawn and measured. The concentration of cholesterol (there was no evidence of precipitation) must have been at least 2,000 mgm. per cent, a figure greatly in excess of that in the blood.

Bile cholesterol. The conclusions drawn from our previous experiments must be modified to the extent that the present experiments show that

TABLE 2
Blood and bile cholesterol

DOG	VOLUME		CHOLESTEROL		BLOOD		BILE CONCENTRATION		TIME
	In	Out	In	Out	Before	End of exper.	In	Out	
	cc.	cc.	mgm.	mgm.	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	hours
10	56.0	18.0	19.6	20.7	158	250	35	115	27
9	11.5	*	23.9	25.1	161	270	208	2000†	23.5

* Less than 1 cc. Contents had to be washed out with saline.

† Approximate figure.

there is always a slightly greater amount of cholesterol recovered than introduced (table 3). In the seven experiments in which the gall bladder may be considered most nearly to approach the normal, the amount of cholesterol recovered exceeded that introduced by not more than 2 mgm. (1.1, 1.3, 1.1, 1.7, 0.9, 1.2, 1.9) for a twenty-four hour period. We cannot confirm the results of Wilkie and Doubilet (3), who found increases of 5 to 36 mgm. of cholesterol in the course of twenty-four hours in what were considered to be normally functioning gall bladders, or of Elman and Taussig (1), and Elman and Graham (7), who also reported increases in the bile cholesterol content. The increase in the amount of cholesterol recovered in our experiments was approximately the same regardless of the amount of cholesterol introduced. For example, 1.1 mgm. in excess was recovered from the gall bladder of dog 13, and 1.2 mgm. from dog 9, although the amounts introduced were very different—for dog 13, 4.6 mgm. and for dog 9, 23.9 mgm. Our data lend little support to those

who believe that a function of the normal gall-bladder wall is to secrete cholesterol into the bile and none to the Wilkie-Doubilet hypothesis that the ratio between the blood and bile cholesterol concentrations determines the direction of the passage of cholesterol across the gall-bladder mucosa.

Microscopic findings. At autopsy all of the so-called normal gall bladders (group I, table 3) except that of dog 9 showed some slight thickening of the wall, but were otherwise normal. The presence of edema of some

TABLE 3
Changes in cholesterol content of hepatic bile in the gall bladder

DOG	VOLUME		CHOLESTEROL			TIME
	In	Out	In	Out	Dif.	
Group I. Normal						
	cc.	cc.	mgm.	mgm.	mgm.	hours
9	11.5	*	23.9	25.1	1.2	23.5
10	56.0	18.0	19.6	20.7	1.1	27.0
11	40.0	9.3	5.0	6.3	1.3	27.5
12	16.0	*	2.6	4.5	1.9	24.5
13	31.0	7.5	4.6	5.7	1.1	23.5
14	40.0	7.0	5.0	6.7	1.7	27.5
15	20.0	8.5	10.6	11.5	0.9	27.5
Group II. Evidences of inflammation or hemorrhage						
16	46.0	8.0	4.1	6.2	2.1	27.0
17	35.0	8.5	5.3	10.2	4.9	28.5
18	20.0	14.5	2.7	11.6	8.9	27.0
19	40.0	9.0	5.4	13.3	7.9	32.0
20	30.0	11.0	6.5	10.3	3.8	25.5
21	53.0	14.0	13.3	17.8	4.5	44.0
Group III. Accessory ducts. Normal mucosa						
22	30.0	11.0	4.5	10.0	5.5	24.0
23	30.0	6.0	4.2	8.0	3.8	24.0
24	25.0	7.0	3.2	8.1	4.9	22.5

* Catheter withdrawn after introducing bile. Less than 1 cc. Contents washed out with saline.

degree in these specimens was confirmed by microscopic sections. The gall bladders in group II showed a greater degree of edema, hemorrhage into the mucosa and submucosa and in some instances areas of mucosal disintegration. In these animals the increase in cholesterol content was considerably greater than when no interstitial hemorrhage was present and the mucosa remained intact.

DISCUSSION. The experiments reported in this paper strengthen the

conclusion drawn from our earlier experiments; namely, that the normal gall-bladder wall of the dog neither secretes nor absorbs cholesterol. A slight amount of cholesterol (1 to 2 mgm. in twenty-four hours) in excess of that introduced was recovered from normally functioning gall bladders which showed no histologic changes except slight edema. If the mucosa normally secreted cholesterol it might also be expected to do this when a physiological saline solution was introduced into the bile-free gall bladder. However, under such conditions only a trace of cholesterol was recovered in the saline solution.

It is possible that the bile introduced into the gall bladder in these experiments may have dissolved 1 or 2 mgm. of cholesterol from the mucosa whereas the saline would not be as good a solvent. Where hemorrhage into the mucosa or submucosa was present considerable cholesterol could be introduced into the bile by the passage of small amounts of blood into the bile. Our data indicate that any cholesterol recovered in excess of the amount introduced into a gall bladder which has not lost its concentrating activity, is dissolved out of mucosa cells, is due to disintegration of these cells, to the passage of small amounts of blood into the bile, or to a combination of these factors.

The finding of very large amounts of cholesterol in excess of that introduced can in every instance in our experience be attributed either to failure successfully to occlude accessory ducts, or to serious injury to the gall-bladder wall, a circumstance which leads to the pouring of fluid into the gall-bladder lumen instead of being absorbed from it. This secreted fluid, as we have previously shown, contains cholesterol (8). Elman and Graham (7) in their paper admit that in at least some of their experiments the bile was diluted rather than concentrated.

Our data do not support Rousselot and Bauman's (4) conclusions that cholesterol is absorbed from the normal gall bladder. Rousselot and Bauman used solutions of cholesterol in bile salt and it is possible that in such solutions cholesterol may not act as it does in the bile. In earlier experiments with a bile salt-cholesterol suspension (2) we also found a very slight loss of cholesterol from such solutions but the loss was never of the magnitude reported by Rousselot and Bauman. In fact the loss was so small as to lead us to conclude that the normal gall-bladder wall neither absorbed nor secreted cholesterol.

There is no evidence in our data that the blood cholesterol concentration influences the cholesterol concentration or the total amount of cholesterol in the gall-bladder bile, or its passage into or out of the gall bladder. Wilkie and Doubilet (3) reported only figures for total cholesterol. Since bile contains only free cholesterol it seemed possible that alterations in the level of the free cholesterol, rather than total cholesterol, might be the determining factor, if the blood cholesterol concentration exerted any

influence on the bile cholesterol concentration. Determinations of free cholesterol showed an even greater difference between the free cholesterol concentration of the blood and the cholesterol concentration of the gall-bladder bile than shown between total cholesterol in the blood and bile. We are at a loss to explain the data reported by these workers.

The data obtained from the present experiments strengthen the conclusions which we drew from our earlier data and indicate more strongly that the normal gall-bladder mucosa does not absorb cholesterol from the gall-bladder bile. Nor has any evidence been obtained which suggests that a function of the normal cholecystic mucosa is to secrete cholesterol into the bile. Its action on the bile cholesterol is similar to that on the bile bilirubin, one of simple concentration.

CONCLUSIONS

1. Cholesterol is not absorbed from hepatic bile placed in the normal bile-free gall bladder.

2. About 1 to 2 mgm. of cholesterol in excess of the amount introduced is recovered after twenty-four hours from hepatic bile placed in the bile-free gall bladder.

3. This excess is most probably the result of the dissolving power of bile on cholesterol in mildly edematous cells.

4. When interstitial hemorrhage has occurred or the mucosa shows disintegration, the excess of cholesterol recovered is in every instance greater than that recovered from the normal gall bladder.

5. When unligated accessory ducts are present, or when fluid is being secreted into the gall-bladder lumen, the amount of cholesterol recovered from the gall-bladder is considerably in excess of that introduced.

6. No relationship has been found in our experiments between concentration of cholesterol in the blood and the concentration of cholesterol in the cholecystic bile.

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THE WORK PERFORMANCE OF ADRENALECTOMIZED RATS MAINTAINED ON A HIGH SODIUM CHLORIDE, LOW POTASSIUM DIET

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Received for publication January 12, 1940

The value of the high sodium chloride, low potassium diet for preventing the overt symptoms of adrenal insufficiency is established (1) (2) (3) (11) although it is recognized that it does not provide a complete substitute for the cortical hormone. I have previously pointed out that the capacity of adrenalectomized rats to perform work is improved by the administration of physiological saline but that it remains at subnormal levels (6). No quantitative data were given. Richter (12) found that the voluntary activity of adrenalectomized rats was improved by a high intake of sodium chloride but remained below normal. Waterman (13) has reported that the level of sodium chloride in the diet of adrenalectomized rats influences their capacity to work.

In the present study I have compared on the basis of work performance 1, rats which had been subjected to sham operations; 2, adrenalectomized rats which were maintained on a stock diet, and 3, rats which were maintained on a diet that was high in its content of sodium chloride and low in its content of potassium.

METHODS. Male rats of the Sprague, Dawley strain which had an initial weight of approximately 180 grams were used. Our stock diet is Purina Dog Chow which has a sodium chloride content of 1.35 per cent and a potassium content of 0.56 per cent. The high sodium chloride, low potassium diet was prepared by mixing the following constituents: casein 36 per cent, sucrose 36 per cent, unsalted butter 7 per cent, lard 18 per cent, dried brewer's yeast 1 per cent, calcium lactate 1 per cent, and cod liver oil 1 per cent. One per cent by weight of sodium chloride was added to this mixture, and the animals receiving this diet were given a 1 per cent solution of sodium chloride to drink.

Bilateral adrenalectomies were performed in the usual manner. No infections occurred postoperatively. For the work tests the animals were anesthetized with phenobarbital sodium. The left gastrocnemius muscle of each animal was weighted with 100 grams and stimulated to

contract three times per second until muscular responsiveness was completely lost. The injections of fluid during work were made subcutaneously at the beginning of the period of work, and again at 6 hours, 24, 30, 48, etc., for the duration of the stimulation. The details of the method have been described (5) (6).

EXPERIMENTS AND RESULTS. In experiment 1 all of the animals were subjected to the work test immediately following the operation. Ten of the animals had false operations and received 5 cc. of water per dose during the period of work; ten were adrenalectomized and did not receive any fluid during the period of work; ten were adrenalectomized and injected with 5 cc. of 0.85 per cent sodium chloride solution per dose; and ten were adrenalectomized and injected with 10 cc. of 0.85 per cent sodium chloride solution per dose. The averages and individual values for work are represented in figure 1. The amounts of work performed by the animals which had been treated with saline were greater, but not significantly so, than for the untreated animals, and all were very small compared to the performance of the control series which possessed their adrenal glands.

In experiment 2, there was a period of delay of seven days between the time of operation and the beginning of the work test. Five rats which had been subjected to false operations were maintained on the Dog Chow diet and received 5 cc. of water per dose during the period of work. Ten adrenalectomized rats were maintained on Dog Chow and did not receive any fluid during the period of work. Thirty adrenalectomized rats were maintained on the high sodium chloride, low potassium diet. During the period of work ten did not receive any fluid, ten received 5 cc. of 0.85 per cent saline per dose, and ten received 10 cc. of 0.85 per cent saline per dose. The values for work are represented in figure 1. The amounts of work performed by the rats maintained on the high sodium chloride, low potassium diet were greater than the amounts of work performed by the adrenalectomized rats maintained on the stock diet, but they were still very small compared to the performances of the control rats. The administration of saline during the period of work did not have any significant effect upon the amounts of work performed.

In experiment 3, there was a period of delay of 14 days between the time of operation and the beginning of the work test. Fifty adrenalectomized rats were fed Dog Chow but 43 of these animals succumbed from adrenal insufficiency before the end of the two week period. Thirty adrenalectomized rats were maintained on the high sodium chloride, low potassium diet. All of them survived the delay period of 14 days. Ten of this group did not receive any fluid during the period of work, ten received 5 cc. of 0.85 per cent saline per dose, and ten received 10 cc. of 0.85 per cent saline per dose. The relative amounts of work performed by

the groups of animals in experiment 3 are similar to those of experiment 2. The values for work are represented in figure 1.

Of 60 adrenalectomized rats which were maintained for one week on the Dog Chow diet, 58 of the animals showed a loss in body weight. In

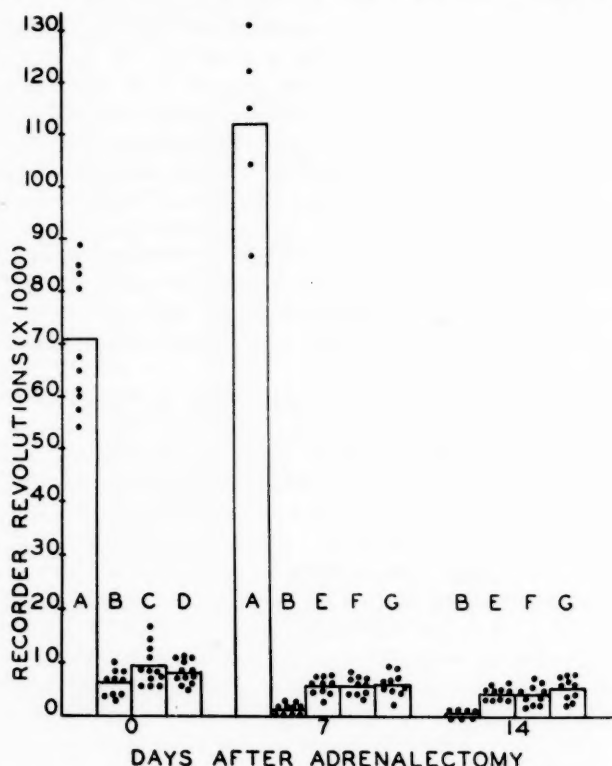


Fig. 1. Work performance of adrenalectomized rats as influenced by diet and by the injection of sodium chloride.

A, sham operated rats—stock diet. B, adrenalectomized rats—stock diet—no fluid during work. C, adrenalectomized rats—stock diet—5 cc. saline per dose. D, adrenalectomized rats—stock diet—10 cc. saline per dose. E, adrenalectomized rats—high Na, low K diet—no fluid during work. F, adrenalectomized rats—high Na, low K diet—5 cc. saline per dose. G, adrenalectomized rats—high Na, low K diet—10 cc. saline per dose.

contrast, of 60 animals which were maintained on the high sodium chloride, low potassium diet, only fourteen showed a loss in body weight at one week. The losses were small. At the end of two weeks only seven rats on the Dog Chow diet had survived and six of this group showed a loss

in body weight, while the remaining rat had maintained exactly its initial weight. At the end of two weeks all of the rats which were maintained on the high sodium chloride, low potassium diet were alive and had gained above their initial body weights. The averages and individual values for body weight are summarized in figure 2.

DISCUSSION. The adrenalectomized rat which is maintained in a state of apparent good health by means of a favorable intake of sodium chloride and potassium is capable of performing more work than is the untreated animal, but is still very inferior to the animal which possesses its own adrenal glands. Ingle, Nilson and Kendall (9) have been able to show that the capacity of the adrenalectomized rat to perform work is not determined by the level of sodium, chloride, or potassium in the blood serum. The distribution of electrolytes in the body fluids must be very closely linked to the function of the adrenal cortical hormone since changes in

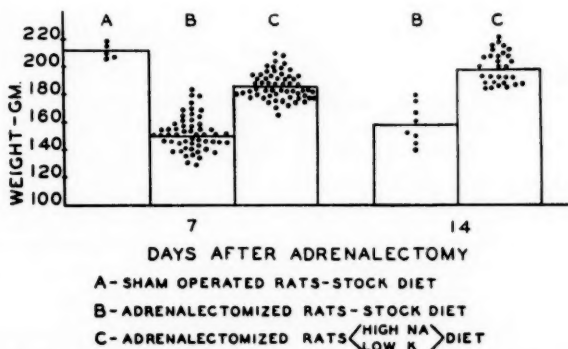


Fig. 2. Body-weights of adrenalectomized rats as influenced by diet

electrolytic balance are produced by either an over-supply or an under supply of cortin (4); the amounts of sodium chloride and potassium taken into the body of the adrenalectomized animal may determine whether it will live or die. However, there is now a large amount of evidence which indicates that the function of the adrenal cortices cannot be fully described in terms of our present knowledge of the metabolism of sodium, chloride and potassium. The capacity of the adrenalectomized animal to resist a stress can be dissociated from the intake of electrolytes and from their levels in the body fluids. Moreover, the effect of the presence or absence of cortin upon carbohydrate metabolism cannot be duplicated by changing the levels of electrolytes in the body fluids (10). The size of the adrenal glands of rats is responsive to either an over-supply of cortin in the body or to an increased functional requirement for cortin by the body (7). Ingle and Kendall (8) found that wide changes in the dietary intake of sodium chloride and potassium had no effect on the size of the adrenal

glands of rats. Similar results were noted by Maes and Freedgood (14) in guinea pigs.

SUMMARY

Adrenalectomized rats which were maintained on Purina Dog Chow showed losses in body weight, a high mortality rate, and a great deficit in capacity to work. Adrenalectomized rats which were maintained on a diet high in sodium chloride and low in potassium all survived, showed gains in body weight and performed more work than did the animals on the Dog Chow diet. However, the work performances of all of the adrenalectomized animals were very small as compared to those of animals possessing their own adrenal glands.

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BODY SIZE AS A FACTOR IN INTERPRETING THE EFFECT OF HORMONE INJECTIONS IN BABY CHICKS

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Received for publication January 13, 1940

The effect of sex hormone injections on the weights of the sex organs is, perhaps, the criterion most widely used in assaying these hormones.

In female fowl, the organs most commonly considered are ovary and oviduct, while in the male these include testes and comb.

In the course of certain hormone investigations at this laboratory, it was noted that body weight exercises a profound influence on the weight of sex organs of baby chicks. Considerable work has been done with hormones on baby chicks, but the importance of this relationship on the interpretation of results of hormone injections on sex organs is generally overlooked. The purpose of this paper is to draw attention to the extent to which the weight of various sex organs depends on body weight.

Review of literature. Souba (1923) found a correlation of 0.521 ± 0.050 between the testis weight and body weight of 39 day-old White Leghorn cockerels. Uselli (1936) reported a correlation of 0.76 ± 0.027 between the live body weight and the testis weight in the Marche breed of cattle. Working with rats, Gayet, Cuny and Quivy (1938) stressed the importance of taking into consideration the weight of sex organs expressed as function of body weight in interpreting the results of sex hormone assays. Kleiber and Cole (1939) state that the endocrine balance of rats depends somewhat on body size.

EXPERIMENTAL MATERIAL. Individually pedigreed Single Comb White Leghorns and Barred Plymouth Rock chicks were sexed and weighed at hatching time, and removed to an electric battery brooder. Chicks were weighed again at seven and seventeen days of age, when they were killed, immediately weighed and dissected. This is the procedure generally followed at this laboratory on hormone experiments with chicks. Ovaries and oviducts in the females and testes in the males were removed and weighed. In addition to these organs, the Bursa Fabricii was dissected and weighed in both sexes.

The rôle of the bursa is obscure in the chicken. Jolly (1915) reported that it reaches the maximum size when the bird is about four months of age. From then on it regresses, and by the time the bird is one year old,

only a vestige of bursa remains. According to Taibel (1933) the removal of bursa in young fowls has no apparent effect on the growth, final weight and sexual maturity. Asmundson et al. (1937) reported that the injection of pregnant mare's serum or of oestrin causes the bursa to regress.

Data obtained over a period of time at this Division seem to indicate that heavy injections of various sex hormones depress the weight of bursa. Consequently this organ was removed as a matter of routine, together with the sex organs.

Tables 1 and 2 present summarized data on the weight of sex organs, bursa and body weight in White Leghorns and Barred Rock chicks.

It is evident from these tables that there is a breed difference in the weights of the testes and bursae.

TABLE 1

Averages of organ and body weights of Single Comb White Leghorn chicks in grams

SEX	NUMBER OF CHICKS	BODY WEIGHTS			ORGAN WEIGHTS		
		Hatching	7 days	17 days	Gonads	Oviduct	Bursa
Male	29	41.2	54.0	92.8	0.022		0.285
Female	35	40.1	53.4	95.9	0.030	0.019	0.306

TABLE 2

Averages of organ and body weights of male Barred Plymouth Rock chicks in grams

NUMBER OF CHICKS	BODY WEIGHTS		ORGAN WEIGHTS	
	7 days	17 days	Testis	Bursa
26	58.6	88.2	0.011	0.084

Both the testes and bursae are smaller in the Barred Rock male chicks than the weight of the corresponding organs in the White Leghorn chicks. The difference is particularly striking in the case of bursa. In Leghorns this organ is more than three times the size of that found in the Barred Rock chicks of the same sex and age. The testes are also larger in the more precocious Leghorn chicks. These differences are statistically significant.

Simple and partial correlation coefficients were calculated, measuring both the degree of association between body and organ weight and the extent to which these associations are affected when each of the variables in turn is held constant. Results of these calculations are summarized in table 3 for White Leghorns and table 4 for Barred Rocks.

One can conclude from these tables that all the organs with the exception of oviduct are highly correlated with the body weight. The bursa shows the highest and oviduct the lowest degree of association. This

high correlation between the bursa and body weight suggests a possibility that the decrease in the weight of bursa as the apparent result of oestrin injection, reported by Asmundson et al. (1937) and observed in this laboratory, is due to a depressing effect of hormone injections on body weight. Breneman (1939) states that the injection of androgens into male Leghorn chicks depressed the body weight.

An interesting observation can be made from the simple and partial correlations testing the degree of association between the organs. The use of the latter statistical method shows that the apparent close correlation between the weights of some organs, as demonstrated by the simple

TABLE 3

Simple and partial coefficients of correlation between body and organ weights in White Leghorn chicks

CORRELATED VARIABLES	SEX	SIMPLE COEF-FICIENT	VARIABLE HELD CONSTANT	PARTIAL COEF-FICIENT
Testis—bursa	♂	0.735*	Body	0.127
Testis—body	♂	0.799	Bursa	0.476
Body—bursa	♂	0.874*	Testis	0.703
Ovary—oviduct	♀	0.075	Body	-0.038
Ovary—bursa	♀	0.508*	Body	0.266
Oviduct—bursa	♀	0.291	Body	0.174
Ovary—body	♀	0.455*	Bursa	0.082
Ovary—oviduct	♀	0.075	Bursa	-0.089
Body—oviduct	♀	0.237	Bursa	-0.003
Body—oviduct	♀	0.237	Ovary	0.229
Bursa—oviduct	♀	0.291	Ovary	0.299
Bursa—body	♀	0.815*	Ovary	0.761
Ovary—body	♀	0.455*	Oviduct	0.451
Ovary—bursa	♀	0.508*	Oviduct	0.509
Body—bursa	♀	0.815*	Oviduct	0.803

* Simple coefficients marked with an asterisk are significant at the 1 per cent point.

correlation coefficients is in reality spurious, when either the body or the bursa weight is held constant.

For example, although the simple correlation coefficient for testis and bursa is 0.735, the coefficient drops to 0.127 when these two organs are compared with the body weight held as a constant variable.

A number of other such apparently significant correlations, their spuriousness masked by the effect of either the bursa or body weight on the interrelationship, are revealed on further examination of table 3. It is interesting to note that in every case when either of these two variables is held constant, the degree of association between the remaining variables is sharply reduced.

On the other hand, the correlation between body and bursa, body and sex organs or bursa and sex organs is undisturbed when either ovary, oviduct or testis weight is held constant.

TABLE 4
Simple and partial coefficients of correlation between body and organ weights in Barred Rock male chicks

CORRELATED VARIABLES	SIMPLE COEFFICIENT	VARIABLE HELD CONSTANT	PARTIAL COEFFICIENT
Testis—bursa.....	0.486	Body	-0.585
Testis—body.....	0.742	Bursa	0.908
Bursa—body.....	0.893	Testis	0.771

All these coefficients are highly significant.

TABLE 5
Regression coefficients showing the regression of the bursa and sex organ weights on body weight

BREED	ORGAN	REGRESSION COEFFICIENT
Leghorns.....	♂ bursa	0.00947
	♀ bursa	0.00819
	Testis	0.00038
	Ovary	0.00031
B. P. Rocks.....	Bursa	0.00024
	Testis	0.00013

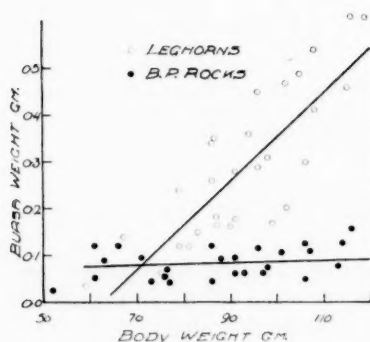


Fig. 1

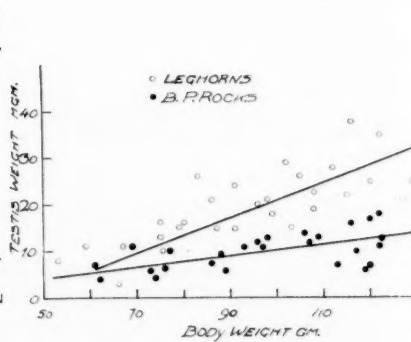


Fig. 2

Fig. 1. Regression of bursa weight on body weight in the 17-day old White Leghorn and Barred Rock male chicks. Note the difference in the slopes of the two regression lines.

Fig. 2. Regression of testis weight on body weight in the 17-day old White Leghorn and Barred Rock chicks.

The regression coefficients still further bring out the relationship between the weights of bursa, testis, ovary and body size. The coefficients are summarized in table 5.

Once more breed differences become apparent, this time in the degree of association between the organs and the body weight. Thus, while for every gram of increase in body weight in the Leghorn male chicks the bursa increases 0.00947 gram, the corresponding increase in the Barred Rock cockerels is only a fraction of that (0.00024 gram). The same holds true for testis, although here the slope of the regression line on the Rock chicks more closely approaches that of the Leghorns. These differences are presented graphically in figures 1 and 2 which show the regression lines of bursa and testis on body weight in the chicks considered in this study.

DISCUSSION. Several points emerge from the analysis presented above. It is self evident that more attention should be paid by investigators to the effect of body size on the weights of sex organs and Bursa Fabricii.

When some of our data, which appeared to show a significant effect of injections on sex organs, were re-examined with this point in mind, it became apparent that what was often considered a significant change in the weight of sex organs was really due to differences in body weights. Organ weights should be corrected to constant body weight. This is particularly important because Breneman (1939), Asmundson et al. (1937) and others reported that the body weight of the sex-hormone treated birds is depressed. Therefore it is possible that the depressions of Bursa Fabricii in the injected chicks, as reported by Asmundson and his co-workers (1937), are due to depressed body weight.

It should be important, therefore, to establish whether this effect is due to a depressed body weight or is a result of the specific action of certain hormones on the bursa. If the latter proves to be the case, then some light would be shed on the physiology of this organ. This point is under investigation at present in this laboratory. In view of the close correlation between the weights of any one of the following three organs, ovary, testis or bursa Fabricii and body size, one must not overlook the depressing effect of sex hormones on body weight in reporting data, particularly when comparing injected and control groups.

Furthermore, some apparently high simple correlations between sex organs are spurious in nature when the data are analyzed by partial correlation.

SUMMARY

1. In chicks the weights of the testis, ovary and Bursa Fabricii are highly correlated with body weight. The oviduct, however, is not significantly correlated with body weight.

2. There is a breed difference in the weight of the testis and Bursa Fabricii in White Leghorn and Barred Rock chicks.

3. Some of the correlation coefficients between organ weights are spurious as revealed by the use of the partial correlation method.

4. Importance of organ and body weight relationship in interpreting results of hormone injections in baby chicks is pointed out.

Acknowledgment. The author wishes to acknowledge his appreciation to Dr. S. S. Munro for helpful advice in the preparation of this paper.

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SOME OBSERVATIONS ON THE NATURE OF THE RESPIRATORY WAVES IN ARTERIAL BLOOD PRESSURE^{1, 2}

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Received for publication January 15, 1940

In the production of respiratory variations in arterial blood pressure a number of factors are involved, among which are changes in heart rate, changes in pressure on the large arteries, changes in the filling of the right atrium and changes in the capacity of the vascular bed of the lungs. It is with the importance of the last factor that we are primarily concerned. The view has been widely held that on inspiration there occurs a dilatation of the lung vessels with a resulting fall in pulmonary and secondarily in systemic arterial pressure. This view has received support from the experiments of Wiggers (1912, 1914) and of Heinbecker (1927), but has been vigorously contested by Cloetta (1912) and by Visser, Rupp and Scott (1924), these workers maintaining that on inspiration the capacity of the pulmonary vessels is decreased and their resistance increased.

Our interest in this problem arose in connection with some experiments planned for another purpose (Nicholson and Trimby, 1940). In these experiments, performed upon dogs anesthetized either with morphine and urethane, or with chloralose, the two main bronchi were individually cannulated. Then, during artificial respiration, ribs 3, 4, 5, 6, 7 and 8 on both sides, including the portion of the sternum to which they were attached were resected as far laterally as the region of the costal angle. About each lung was then carefully placed a metal container, a tight seal about the pedicle being obtained by means of a rubber dam and vaselined gauze. After this had been done artificial respiration was carried on by rhythmically withdrawing air from the lung containers, the inflation of the lungs occurring as in natural ventilation as the result of a decrease in pressure on their external surfaces. The ventilation was ordinarily sufficient to produce apnea. No changes in heart rate were observed. No contractions of the abdominal muscles or diaphragm occurred. The

¹ Preliminary Report—Proceedings, American Physiological Society, This Journal **123**: 205, 1938.

² These experiments were supported by a grant from the Rockefeller Foundation to Robert Gesell for the studies of respiration.

pressure changes responsible for respiration could not affect the large arteries or veins of the thorax or the heart itself. Changes in the pulmonary vascular bed represented the only mechanism now capable of producing rhythmic blood pressure variations synchronous with respiration. Nevertheless under these conditions well marked respiratory waves in blood pressure invariably occurred as illustrated in figure 1.

Since the pumps ventilating the two lungs were operating at slightly different rates the blood pressure waves rhythmically appear and disappear, the waves being greatest when ventilation of the two lungs is in phase and being least or disappearing completely when inspiration in one lung coincides with expiration in the other. Examination of figure 1 shows that the fall in mean blood pressure begins shortly after the beginning of inspiration and extends somewhat into expiration, representing a lag of about one or at most two heart beats. This relationship is one commonly observed during natural ventilation. In the experiment illustrated



Fig. 1. Artificial ventilation by negative pressure. Upper two records spirometer tracings from right and left lungs respectively, upstroke inspiration. Lower record mean blood pressure by mercury manometer from cannula in femoral artery. Numerals indicate synchronous points, odd numbers the troughs of the blood pressure waves, even numbers the crests.

the heart rate respiratory rate ratio is about 5:1, a common ratio in man. This type of respiratory blood pressure wave was not altered by vagotomy.

The fact that respiratory waves in blood pressure of considerable magnitude and of apparently normal type occur when all mechanisms capable of producing such waves except changes in pulmonary blood flow have been eliminated of course does not indicate that these other factors are not involved in the production of such waves under normal conditions. It does, however, strongly suggest that under normal conditions changes in the capacity of the pulmonary vascular bed may play a rôle of considerable importance in the production of such waves.

It should be possible to settle the question as to whether the capacity of the pulmonary vascular bed is increased or decreased during inspiration by comparing the effects on blood pressure of inflating the lungs by positive and negative pressure. Provided the pressure changes are confined to the lungs it appears that a reversal of the relationship existing between the blood pressure changes and the respiratory phases on changing from

positive to negative pressure could safely be taken to indicate an increase in vascular capacity on inflation by negative pressure and a decrease on inflation by positive pressure. Visscher, Rupp and Scott (1924), the chief proponents of the view that the capacity of the lung vessels decreases on inflation strongly insist that the effects must be the same whether inflation is by negative or positive pressure and it is certainly difficult to imagine an increase in capacity on inflation by positive pressure and a decrease on inflation by negative pressure.

In our experiments, after the metal containers had been placed about the lungs it was possible to carry on artificial ventilation either by rhythmically forcing air into the lungs through the bronchi or by rhythmically decreasing the pressure on the external surfaces of the lungs. In either case the pressure changes were confined to the lungs. A change from one to the other type of ventilation could be made within a few seconds simply by turning a few valves on the pumps. Such a change invariably

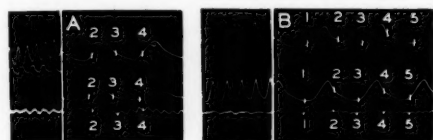


Fig. 2. A. Artificial ventilation by negative pressure. B. Artificial ventilation by positive pressure. Upper two records spirometer tracings from right and left lungs respectively, upstroke inspiration. Lower record mean blood pressure by mercury manometer from cannula in femoral artery. Numerals indicate synchronous points, odd numbers the troughs of the blood pressure waves, even numbers the crests.

was found to reverse the relationship which blood pressure changes bore to the phases of respiration. During inflation with negative pressure inspiration caused after a brief lag a fall in mean pressure, expiration causing a rise. If the inflation was by positive pressure inspiration was followed after a similar lag by a rise in pressure, expiration by a fall. The amplitude of the blood pressure waves was usually somewhat less when positive pressure was used. These differences are illustrated in figure 2. In record A inflation is by negative pressure, in record B from the same animal it is by positive pressure. It is seen that the even numbers marking the crests of the waves occupy almost exactly the same positions on the respiratory waves in record A as do the odd numbers marking the wave troughs in record B. The pulse rate respiratory rate ratio in this particular experiment was about 6:1 in the case of negative pressure compared to 7:1 in the positive pressure record. Such a slight difference could certainly not account for the complete reversal shown.

The evidence appears quite strong, then, that during inflation of the

lungs by a decrease in the pressure on their external surfaces there occurs an increase in the capacity of the vascular bed of the lungs. This doubtless plays some part in producing the fall in blood pressure commonly observed during inspiration. It is also, as pointed out by Wiggers (1939), of considerable importance in delaying the appearance of the rise in systemic blood pressure resulting from increased filling of the right atrium during inspiration.

SUMMARY

When artificial ventilation was accomplished by rhythmically decreasing the pressure on the external surfaces of the lungs without altering the pressure on other thoracic structures well marked respiratory waves in blood pressure were observed, characterized after a slight lag, by an inspiratory fall and an expiratory rise in pressure as commonly seen with natural ventilation. Artificial ventilation produced by rhythmically forcing air into the lungs through the bronchi reversed these effects. It is assumed that inspiration produced by decreasing the pressure on the external surfaces of the lungs results in an increase in the capacity of the vascular bed of the lungs. This is believed to be partly responsible for the inspiratory fall in blood pressure normally seen and also to be important in delaying the rise in pressure resulting from increased filling of the right atrium during inspiration.

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THE ACTION OF OSMOTICALLY-ACTIVE SUBSTANCES ON THE HEART RATE

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Received for publication January 16, 1940

Such substances as glucose and sucrose in sufficient concentration (0.05 molar or more) cause a slowing of the cold-blooded heart. I have recently suggested that these substances act by virtue of their osmotic activity (Spealman, 1938). In the present experiments I have studied the action of urea and of glucose on the dogfish-heart rate and the action of several compounds, similar in nature but of different molecular weight, on the frog-heart rate in an attempt to obtain evidence that only those substances which are osmotically active (because of their higher molecular weight) cause a slowing of the heart rate.

EXPERIMENTAL. In the experiments with the dogfish heart the sinus venosus, usually with part of the atrium attached, was removed and suspended by a thread in a solution containing 0.5 molar urea in 50 per cent sea water. This solution will be referred to as normal solution. (All solutions were made up of unconcentrated sea water plus ordinary tap water containing the necessary concentration of urea.) After allowing time for the preparation to become stable, the experiment was begun. All experiments were carried out at room temperature. Two heart rate values were obtained at five-minute intervals with the heart in normal solution; two values were then obtained in the same manner with the heart in the experimental solution; and finally, two more values were obtained with the heart again in the normal solution. The average of the two values obtained in the experimental solution was divided by the average of the four values obtained in the normal solution; the resulting figure is the one used as an expression of the heart rate.

The following studies were made on the dogfish-heart rate: the effect of variation of the sea water concentration of the normal solution, the effect of variation of the urea concentration of the normal solution, and the effect of addition of glucose to the normal solution.

In the experiments with the frog heart, a similar procedure was followed. Ringer's solution was used as the normal solution. Because I wanted a higher degree of accuracy in the frog heart experiments, the heart rate was

followed over a much longer period of time, usually about one hour in each solution. The heart rate values were then plotted against time and an average heart rate value was obtained by drawing a smooth curve through these points. Otherwise these experiments were similar to those on the dogfish-heart.

TABLE 1

Summary of the data on the dogfish heart showing the heart-rate values obtained with various solutions

In all cases the heart rate is expressed as a decimal fraction of the heart rate in normal solution. *S* indicates the heart stopped beating.

a. Heart-rate values in solutions containing various concentrations of sea water (urea concentration, 0.5 M/L)

CONCENTRATION OF SEA WATER per cent	HEART RATE		
	Expt. 1	Expt. 2	Expt. 3
75.0	0.92	1.05	0.95
62.5	1.04	1.01	
50 (normal)	1.00	1.00	1.00
37.5	S	S	0.96
25.0	S	S	

b. Heart-rate values in solutions containing various concentrations of urea (sea water concentrations, 50 per cent)

UREA CONCENTRATION M/L	HEART RATE		
	Expt. 1	Expt. 2	Expt. 3
1.00	S	0.92	0.91
0.75	1.02	0.94	
0.50 (normal)	1.00	1.00	1.00
0.38	0.94	1.07	
0.25	S	0.90	S

c. Heart-rate values in normal solution containing glucose

GLUCOSE CONCENTRATION M/L	HEART RATE				
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
0.30	0.63	0.80	0.86	0.81	0.78

The effect on the frog-heart rate of 0.1 molar concentrations of ethyl alcohol, urea, glycerol, propylene glycol, arabinose, glucose, mannitol and sucrose in Ringer's solution was studied (table 2).

Also experiments were made with the frog-heart ventricle to find which of these substances were able osmotically to remove water from the tissue.

The procedure consisted of weighing the ventricle first in normal Ringer's solution, then in Ringer's solution containing a 0.2 molar concentration of one of the above substances and, finally, again in normal Ringer's solution. (The reason for using 0.2 molar instead of 0.1 molar solutions here is that it is easier to demonstrate any change in weight with the higher concentration.) Several weighings were made over a period of an hour or more for each solution. The weight of the ventricle in each case is expressed as a decimal fraction of its weight in normal Ringer's solution (table 2).

RESULTS. The data on the dogfish heart are summarized in table 1. Within certain limits, the heart rate is not significantly changed by varia-

TABLE 2

Summary of data obtained on the frog heart showing the effect of various substances on the heart rate and on the weight of the ventricle

The molecular weights of the compounds studied are given in the second column. Heart rates and ventricular weights are expressed as decimal fractions of the values found in normal Ringer's solution. The figures following the (average) heart rate values are the mean deviations. One-tenth molar concentrations were used in the heart rate studies and 0.2 molar concentrations in the weight studies.

SUBSTANCE ADDED TO NORMAL RINGER'S SOLUTION	MOLECULAR WEIGHT	HEART RATE (EACH FIGURE IS AN AVERAGE OF VALUES OBTAINED ON 6 HEARTS)	WEIGHT OF VENTRICLE (EACH FIGURE IS AN AVERAGE OF VALUES OBTAINED ON 2 HEARTS)
1. Ethyl alcohol.....	46.05	0.99 \pm 0.06	1.00
2. Urea.....	60.05	0.98 \pm 0.05	1.00
3. Glycerol.....	92.06	0.97 \pm 0.04	0.98
4. Propylene glycol.....	76.06	0.95 \pm 0.02	0.98
5. Arabinose.....	150.08	0.84 \pm 0.01	0.93
6. Glucose.....	180.09	0.76 \pm 0.02	0.89
7. Mannitol.....	182.11	0.75 \pm 0.06	0.87
8. Sucrose.....	342.17	0.73 \pm 0.07	0.86

tion of the sea water concentration of the normal solution (1a) or by variation of the urea concentration of the normal solution (1b). Some of these solutions caused the heart to stop; apparently these solutions were too toxic to allow the heart to function properly. The heart rate is lowered, and to about the same extent in different experiments, by the addition of glucose to the normal solution (1c).

The data on the frog heart are summarized in table 2. Ethyl-alcohol, urea, glycerol, and propylene glycol cause no apparently significant change in the frog-heart rate. Arabinose, glucose, mannitol, and sucrose lower the heart rate to about the same extent. The former four substances cause no change in the weight of the ventricle; the latter four substances cause a decrease in ventricular weight, presumably by osmotic transfer of water.

DISCUSSION. Glucose causes a decrease in the dogfish-heart rate; however, variation of the concentration of urea, a substance which is freely permeable to dogfish tissues (see Smith, 1931) causes no change in the heart rate. This is to be expected if the factor responsible for the slowing is the osmotic transfer of water. Variation of the sea-water concentration does not affect the heart rate, contrary to what might be expected if the osmotic pressure is the sole factor involved here. This result is analagous to that I have previously obtained with the frog heart when the sodium chloride concentration of the Ringer's solution was varied. I have already offered a possible explanation for this (Spealman, 1938).

The substances studied on the frog heart fall into two groups: those that neither significantly affect the heart rate nor cause any change in ventricular weight and those that decrease the heart rate and also cause a loss of weight of the ventricle. This indicates that, of the substances employed, only those that are osmotically active cause a slowing of the heart. It is also significant that the substances which slow the heart do so to about the same extent, for the concentration of the experimental substance was the same in each case.

SUMMARY

1. Within certain limits variation of the urea or sea water concentration of the solution causes no change in rate of the isolated dogfish heart; further variation of the concentration of these substances may cause the heart to stop. Addition of glucose to the solution decreases the heart rate.

2. Ethyl alcohol, urea, propylene glycol, and glycerol in the concentration used (0.1 molar) cause no change in the frog-heart rate; however, arabinose, glucose, mannitol, and sucrose in the same concentration decrease the heart rate. Evidence obtained on the ventricle indicates that the former substances are osmotically inactive while the latter substances are osmotically active.

Acknowledgments. I am indebted to Prof. R. Legendre, director of the Laboratoire Maritime du Collège de France, for his sympathetic interest in my research and for his many kindnesses.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY
FIFTY-SECOND ANNUAL MEETING

New Orleans, Louisiana, March 13, 14, 15, 16, 1940

Observations on reactive hyperemia in various portions of the extremities.

DAVID I. ABRAMSON and EUGENE B. FERRIS, JR. (by invitation).

May Institute for Medical Research of the Jewish Hospital and Department of Internal Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

By means of appropriate plethysmographs, the effect of varying periods of arterial occlusion upon blood flow in the hand, forearm and leg was studied in a series of normal subjects. The experiments were performed under different bath and environmental temperature conditions.

It was found that in the hand, the reactive hyperemia produced by a ten minute occlusion was generally sufficient to repay only about 10 to 20 per cent of the theoretical oxygen debt incurred. During reflex vasodilatation, when the control flow was higher, even less was repaid. In most instances, the initial increase in hand flow during the period of reactive hyperemia was followed by a negative phase in which the flow dropped below the control level. This occurred simultaneously with the onset of a sensation of burning and tingling in the fingers.

In the forearm and in the leg, a 10 minute arterial occlusion was generally followed by an increase in flow which equaled or more than compensated for the blood flow debt. The only exception occurred when the blood vessels were initially reflexly dilated, in which case the blood flow debt was not entirely rapid. The negative phase, observed in the hand, was not present in the forearm or leg. These responses are similar in some respects to those reported for the sympathectomized hand.

Comparison between resting blood flow at a bath temperature of 45° with the maximal reading obtained during reactive hyperemia, at a bath temperature of 32°, revealed that in the hand the application of local heat is always a more potent stimulus than tissue anoxia, while in the forearm and leg, the reverse is true.

The above findings are in accord with the view that the blood flow to the forearm and leg, under ordinary environmental conditions, is closely related to the metabolic requirements of the tissues, while that to the hand is dependent primarily upon the need for body heat dissipation.

Variability and error in cardiac output estimation. WRIGHT ADAMS and

IRENE SANDIFORD (introduced by R. W. Gerard). Department of Medicine, University of Chicago, Chicago, Illinois.

The usefulness of the Grollman acetylene method and its various modifications is limited because it is impossible to distinguish between technical error and physiologic variation when divergent results occur. By introducing a quickly acting gate valve between the bag and the sampling tubes

at the mouthpiece it is possible to take multiple samples of alveolar and bag gas at the end of expiration with the valve closed. In satisfactory determinations calculations of arteriovenous oxygen difference from successive samples from each source yield values in close agreement which remain constant for two or more breaths. This modification reveals and usually identifies the cause of errors due to incomplete lung bag equilibrium, recirculation of acetylene containing blood to the lungs, leaks in the lung bag system or sampling tubes, and inaccurate gas analysis.

With this method it is possible to set some desired degree of accuracy as the basis for accepting or discarding results. In eighty-two determinations on five subjects, the error in the estimation of arteriovenous oxygen difference resulting from the above-mentioned causes was five per cent or less in thirty-eight cases, from six to ten per cent in thirty-nine cases and more than ten per cent in five cases. By discarding the results subject to large error it is possible to identify discrepant results due to physiologic variation.

Morphine-atropine antagonism in relation to propulsive activity in the dog's colon. HARRY F. ADLER (by invitation), L. R. KRASNO (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Following the subcutaneous administration of morphine, one of the most consistent results obtained by various investigators has been an increase in intestinal tone. We have found this exaggerated tonus and motility of the dog's colon to endure for several hours after administration of $\frac{1}{4}$ grain of morphine. In order to study the relation of this exaggerated tonus and motility to its propulsive qualities, we have devised an apparatus for measurement of propulsion. This apparatus consists of a balloon and tube which is placed in the colon of unanaesthetized, trained animals through an appendicostomy. The balloon is connected to a water manometer in order to record circular motility. A string attached to the junction of the tube and balloon is led to a calibrated spring and tambour system in such a way that motility tending to shift the balloon to a lower level of the colon will affect the tambour, and thus a kymograph lever.

In twenty experiments upon four dogs circular tonus and motility of the colon were seen to increase within two minutes after morphine injection and remain exaggerated for several hours. During this period motility of the propulsive type varying from a few grams to 130 grams was recorded. Twenty more experiments were conducted in which the animals were atropinized (1.1 mgm.) before giving the morphine. Atropine was found to have a profound effect upon propulsive activity; however, in the dosage used, that circular activity, which is nonpropulsive in nature, was not significantly reduced.

Water load and blood dilution in dogs. E. F. ADOLPH and H. D. KINGSLEY (by invitation). Department of Physiology, University of Rochester, Rochester, N. Y.

New data were obtained to answer the old question, Is the blood appreciably diluted during water diuresis? Water load is excess or deficit of water in the whole body. Venous blood dilution is computed as $(C_0/C_1) - 1$, from measurements of: dry residue of plasma and of blood, refractive

index of plasma above that of water, chloride of plasma, hemoglobin, and hematocrit. Urine was collected continuously from exteriorized bladder in 23 tests on 2 dogs weighed every 15 minutes. Water was administered by stomach *a*, in one dose 6.1 per cent of the body weight, or *b*, in ten doses, each 1.0 per cent, at 15-minute intervals. Or *c* water was withdrawn on constant diet until the body weight was -4.0 per cent, then 6.0 per cent was voluntarily drunk. Control measurements, *d*, were made both before each test and in separate tests.

In series *a* dilution appeared within 10 minutes and was maximal at 60 minutes; then urine output was also maximal. In both *a* and *b*, plasma dilution was after 60 minutes twice as great as whole body dilution or load; or, the volume of distribution of the water excess was equivalent to half the body weight.

Correlation coefficients were ascertained between 50 observations of plasma dilution, at least 60 minutes after administration, and water load ($r +0.66$), and rate of water excretion ($+0.48$). Mean dilutions were thrice the standard deviation, and 13 times the standard error, of controls.

In water deficit, *c*, the load was distributed in about two-thirds the body weight. Upon drinking, plasma was often further concentrated within 15 minutes; thereafter dilution progressed more slowly than in *a*, indicating possible lag in water absorption or rapid unloading in liver or elsewhere. The dilution was maximal at 90 minutes, when, during distinct diuresis, the load was distributed to one-third the body.

Conclusions: 1, *plasma* solids are always diluted when large water excesses prevail; 2, rates of water excretion are *highly* correlated with positive water loads ($r +0.69$) and with plasma dilutions ($+0.48$); 3, dilutions of plasma appear *later* if following water deficit than if following no load; 4, *distributions* of water excesses are ultimately similar after all three types of water administration.

Cyclopropane and parasympathetic action in reptilian and amphibian hearts.

JOHN ADRIANI and E. A. ROVENSTINE (introduced by Stevens J. Martin).

Division of Surgery, Department of Anesthesia, New York University College of Medicine, New York, N. Y. (Read by title.)

Stimulation of the parasympathetic division of the autonomic nervous system by cyclopropane has been suggested from observations made during clinical anesthesia. The following experiments were performed to support this contention:

Forty observations were made on 12 turtle and 3 frog vagotomized hearts, isolated and in situ, which were perfused with Ringer's solution at 20°C. and arranged to record ventricular contractions. Ten seconds after Ringer's solution, one-fifth saturated with cyclopropane, was perfused through the heart, a slowing and decrease in the amplitude of the ventricular contractions occurred. Eserinized preparations similarly perfused showed immediate asystole with the heart in diastole. Control conditions were restored following both procedures upon perfusion with plain Ringer's solution in 2 and 10 minutes respectively. These effects were identical to those noted upon vagal stimulation or after acetylcholine (0.01 mgm.) was added to the perfusate. Atropine sulphate 1:10,000 added to the perfusion prevented these effects or restored the control rhythm.

In some eserinized preparations, after asystole was produced by cyclo-

propane-Ringer's perfusion, the perfusate was removed, the gas completely exhausted and the solution then perfused through a second eseriniz heart. Cardiac slowing or asystole occurred. These effects were removed with atropine sulphate perfusion. In control studies the stock cyclopropane-Ringer's solution was exhausted of the gas and then perfused through an eseriniz heart. No slowing or asystole resulted. Furthermore, Ringer's solution saturated with ethylene or ether, used instead of cyclopropane, produced no cardiac slowing.

These experiments suggest that cyclopropane stimulates the parasympathetic division of the autonomic nervous system and that a substance like acetylcholine may be liberated. The absence of the response with ethylene and ether indicate a specific action of cyclopropane.

The effect of decerebration on cardiac arrhythmias produced by adrenalin in dogs under cyclopropane. CHARLES R. ALLEN (by invitation) and WALTER J. MEEK. Department of Physiology, University of Wisconsin, Madison.

Ventricular extrasystoles and tachycardias of 50 to 70 seconds duration, as shown by continuous electrocardiograms, were induced by the intravenous injection of adrenalin in dogs whose hearts were predisposed to the development of irregularities by deep cyclopropane anesthesia. These dogs were then decerebrated under the same concentration of cyclopropane and injected with adrenalin as before. No irregularities occurred. Higher concentrations of adrenalin produced some arrhythmias but definitely showed protection of the heart from the full effects of the dose.

The dogs were decerebrated by a modification of the anaemia method of Pollock and Davis (J. Comp. Neurology 50: 377, 1930). The decerebrations usually were done in two stages. In the first stage the basilar artery was ligated and the dogs were allowed to recover for several days so that the effects of trauma such as increased intracranial pressure and hemorrhage might disappear. Decerebration was then produced by clamping the carotids. After at least 30 minutes of cerebral anaemia the carotids were released in order to allow the carotid sinuses to function at the time of the adrenalin injection.

It is believed that we have shown a center of anesthetic action in producing cardiac irregularities under cyclopropane anesthesia to be located in the brain anterior to the pons. The evidence is that decerebration protects the heart from the arrhythmias ordinarily seen on adrenalin injection when the whole brain is functioning. The fact that higher concentrations of adrenalin do produce irregularities after decerebration is interpreted as indicating other points of action, probably, the myoneural junctions of the sympathetic nerve endings in the heart.

On the penetrability of lymphatic endothelium. LANE ALLEN. Department of Anatomy, University of Georgia School of Medicine, Augusta. (Read by title.)

These experiments were designed to determine the size of the openings through which particles placed in the peritoneal cavity reach the diaphragmatic lymphatics.

In the rabbit the diaphragm was exposed and covered with a suspension of powdered silica. The maximum size of particles observed histo-

logically in the lymphatics was 1.5 microns. Measurements were also made on frog erythrocytes which, after injection into the peritoneal cavity, were found in histological sections to be passing through the peritoneum and underlying lymphatic endothelium. These showed the peritoneal stomata to be from 4 to 7 microns in diameter which is within the range of size of stomata demonstrable by silver impregnation. Measurements on the same frog erythrocytes passing through the intercellular cement of the lymphatic endothelium deep to the peritoneal stomata showed that the peritoneal stomata were from about one-half to one micron in diameter.

Since we were able to find many more endothelial stomata per unit area by plugging them with frog erythrocytes than can be demonstrated by the silver technique it is concluded that endothelial stomata are not constant openings. It seems evident that with each expiratory movement of the diaphragm the pressure differential resulting from the coincident increase in volume of the lymphatics breaks the intercellular cement to allow for the passage of particles of about the same diameter as the intercellular cement.

Toxicity of high salt intake in adrenalectomized rats. EVELYN ANDERSON, MICHAEL JOSEPH (by invitation), VIRGIL HERRING (by invitation) and HERBERT M. EVANS. Institute of Experimental Biology, University of California, Berkeley.

There is an optimal intake of NaCl for adrenalectomized rats which restores for a time the deranged functions of carbohydrate and electrolyte metabolism. Adrenalectomized rats given 1 per cent NaCl store fed carbohydrate as follows: 15th day post-adrenalectomy liver glycogen 1074 mgm. per cent; 20th day, 1056 mgm. per cent; 30th day, 662 mgm. per cent. The corresponding value for adrenalectomized rats not given NaCl: 15th day, 432 mgm. per cent; for controls with adrenals intact, 1977 mgm. per cent. Urinary excretion of Na and K is normal in rats given 1 per cent NaCl on the 48th day post-adrenalectomy. If twice the optimal dose of NaCl is given the survival period is the same as for untreated adrenalectomized rats. On the 15th day post-adrenalectomy the liver glycogen is 630 mgm. per cent; normal controls, 1202 mgm. per cent. When the optimal dose of NaCl is tripled adrenalectomized rats die in 24 hours.

The positive phase of the "injury action potential." RICHARD ASHMAN, WALTER S. WILDE (by invitation) and NORMAN WOODY (by invitation). Department of Physiology, Louisiana State University School of Medicine, New Orleans.

It has recently been shown by Eyster, Meek, Goldberg and Gilson (Am. J. Physiol. 124: 717, 1938) that the monophasic action current from ventricular muscle not only represents the disappearance of negativity at the injured surface, but consists also of a positive phase, i.e., the injured surface becomes positive relative to the potential of resting muscle. We have confirmed this observation and have done further work indicating that this positive phase may readily be explained in terms of the polarized membrane theory.

1. The monophasic curve from the turtle ventricle, recorded with bipolar or unipolar derivation, possesses a very small positive phase when the record is taken immediately after injury by crushing or cutting.

2. *Pari passu* with the temporal decrease in intensity of the current of injury, there is an increase in the relative size of the positive phase.

3. Within approximately a minute after induction of injury the monophasic curve becomes impure. The impurity, shown by an initial spike, a descent from the spike, and a later rise before the final descent, is due to partial response at the injured surface. This can be proved by local cooling or warming at the injury which respectively lengthens or shortens the duration of the depression following the spike.

4. Stimulation of the injured surface just after impurity becomes manifest, e.g., by salt crystals, yields a curve in which the monophasic response is preceded by a sharp downward deflection (negativity) and the spike is greatly reduced or absent.

These facts point to the existence at the injured surface, of a partially polarized membrane which fails to respond at first after the induction of injury. The presence of this persistently polarized membrane sufficiently accounts for the positive phase and the postulation of some other mechanism is unnecessary. In this connection a recent paper is pertinent (Proc. Soc. Exper. Biol. and Med. 42: 17, 1939).

Factors influencing the early action of estrogen upon the uterus. E. B. ASTWOOD. Department of Obstetrics, Johns Hopkins University and Hospital, Baltimore, Md.

The increase in uterine weight and tissue water during the six hours following an injection of estrogen has proved a satisfactory criterion for the assay of estrogenic substances. Factors which may modify this reaction have been studied to determine the conditions which may alter the accuracy of the test and to approach a clearer understanding of its mechanism. The methods employed were similar to those previously published (Endocrinology 23: 25, 1939), immature rats were given a single small dose of estradiol and then subjected to various additional influences.

The route of administration, whether subcutaneous, or intravenous, and the volume of the vehicle up to 1 cc., whether oil or water, did not appreciably influence the time relation of the response. The high level of uterine water was reached in each case after six hours and fell to a low level in twelve hours. This fall could be prevented by a second injection given six hours after the first, indicating that estrogen is rapidly mobilized and is largely inactivated or excreted within six hours. Toxic agents had little influence on the response until the M.L.D. was approached and then inhibition occurred. Moderate hydration or dehydration were without noticeable effect; marked hydration caused an increased effect which was proportional to the general increase in body weight; severe dehydration by injection of 20 per cent glucose prevented uterine swelling. Esserin, Pilocarpine and Prostigmine in maximal dosage caused no augmentation of the estrogen response. Atropine caused no inhibition until the toxic dose had been considerably exceeded. It is inferred from this that acetylcholine is not an essential mediator of the response.

Progesterone, while causing a slight increase in uterine weight in the absence of estrogen, produced a distinct inhibition of the estrogen effect. The inhibition of uterine hydration was more marked than the inhibition of weight increase and the degree of antagonism was related to the ratio

of the two hormones. Testosterone was not inhibitory; given alone it evoked a considerable weight increase. In the assay of estrogen the only likely factor introducing an appreciable error is the presence of large amounts of contaminating androgen or progesterone.

The nervous gradation of muscular contraction. A. KEARNEY ATKINSON (by invitation), RICHARD C. BROWN (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor

Action potentials of the exposed internal intercostal muscles of the dog were recorded with floating electrodes during progressive hypercapnia. With increasing strength of inspiratory contractions, motor units inactive during eupnea became active. Initial activity of each new unit made its appearance in the latter part of the inspiratory period when contraction was at its maximum. As hyperpnea progressed such recruited motor units fired earlier and earlier in the inspiratory phase and accelerated progressively to higher and higher frequencies up to the end of inspiration. The number of twitches per fiber, per inspiration, and the maximum frequency of twitch, therefore, increased with increasing chemical drive. Plotting the number of twitches per fiber, per inspiration, against increasing drive showed a rapid initial increase in activity, followed by a more slowly increasing activity which tended to approach a steady state response. The curve of maximum frequency of twitch had a similar contour. Each unit reached a maximum of approximately 20 discharges per second shortly after coming into activity. The result of these two adjustments to increasing chemical stimulation was an ever increasing sum total of muscle fiber twitches which paralleled the depth or strength of inspiration.

Similar studies were made on the exposed diaphragm during open pneumothorax and after double vago-section. Recruitment of additional motor units was found to occur in the same manner as described for the intercostal muscles. The number of twitches per fiber per inspiration and the maximum frequency of fiber twitch, however, showed a less marked tendency to reach an early maximum. The maximum frequency of twitch for diaphragmatic muscle units was approximately 40 per second or about twice that of the intercostal muscle.

Recruitment of new active units and the associated increase in the sum total of fiber twitches seemed to be the main factors grading contraction. On the other hand gradation through changes in frequency of twitch seemed of secondary importance.

*Sensory cortex of the chimpanzee.*¹ PERCIVAL BAILEY (by invitation), J. G. DUSSER DE BARENNE, HUGH W. GAROL (by invitation) and W. S. McCULLOCH. Laboratory of Neurophysiology, Yale University, School of Medicine, New Haven, Conn.

By local strychninization and recording electrical activity of the cerebral cortex its sensory region and the functional organization thereof were investigated in the chimpanzee.

I. The location and extent of the sensory cortex of the chimpanzee is similar to that of the macaque. It occupies a large region before and behind the fissura centralis.

¹ Aided by a grant from the John and Mary R. Markle Foundation.

Local strychninization of any area immediately outside the sensory cortex thus delimited does not produce strychnine-spikes in its electrogram, i.e. does not "fire" any area within it.

II. The similarities of the functional organizations of the sensory cortex of the chimpanzee and the macaque are: 1, the existence of functionally discrete leg-, arm- and face subdivisions; 2, area 6 "fires" area 4; 3, area 4 does not "fire" area 6; 4, between areas 4 and 6 lies a region whose local strychninization gives suppression of the electrical activity within the sensory cortex; 5, area 4 "fires" itself and the postcentral cortex; 6, the postcentral cortex "fires" area 4; 7, there is a postcentral region whose local strychninization gives suppression of electrical activity within the sensory cortex.

The dissimilarities are: 1, the "firing" within the area strychninized is much more restricted; 2, the "firing" of areas functionally related to the one strychninized is more restricted in distribution and amplitude of the strychnine-spikes; 3, the suppressions of electrical activity obtained in the chimpanzee are more prolonged and more widespread.

The direct current thermostromuhr. E. J. BALDES and J. F. HERRICK. Divisions of Biophysical Research and Experimental Medicine, The Mayo Foundation and The Mayo Clinic, Rochester, Minn.

At a previous meeting a report was given on the analysis of the Rein thermostromuhr as used for measuring blood flow. As a consequence of this analysis, a new direct current thermostromuhr was described and demonstrated. Our experience for the past three years has indicated that the direct current thermostromuhr is reliable and useful for the measurement of blood flow in the trained dog.

An attempt will be made to elucidate the following factors pertinent to the use of the thermostromuhr; namely effect of thickness of wall of blood vessel, relative position of thermojunctions, effect of turbulent and pulsating flows and causes of variation in calibration.

The reliability of the method will be shown by the comparison of simultaneous measurements of blood flow using the thermostromuhr and a flowmeter.

Reflex intracellular water increase in cold environments. HENRY G. BARBOUR. Department of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Conn.

In the reflex response to cold the blood of mammals concentrates apparently with respect to all solid components. Since the osmotic concentration was found equal to the increase indicated by specific gravity in cats (and later in monkeys) Gilman and I suggested that the acute movement of water must be accounted for by its attraction to an accumulation of metabolites in the cells.

This hypothesis has been tested by comparison of extra- and intracellular water content of normal cats with that of cats killed after one-half hour in a room at 25°F., 35°F. or 45°F. The method of chloride and water determination in serum and tissues has been employed.

Water in seven normal cats ground up without the skin was found to be from 52 to 64 per cent intra-cellular; in seven cold room cats it varied from 61 to 70 per cent. Calculated for the whole cat including the

skin the normal cats ranged from 41 to 56 per cent, whereas the cold room cats ranged from 51 to 66 per cent. No significant differences have been found in these water shifts between exposures (with shivering) to 25°F and (without shivering) to 45°F. It is concluded that the principal factor in the reflex shift of water in response to a cold environment is attraction to the interior of the cells.

*Colorimetric determination of lactic acid.*¹ S. B. BARKER. The New York Hospital and the Department of Medicine, Cornell University Medical College, New York City.

Colorimetric lactic acid determinations have been extensively studied in order to evolve a satisfactory procedure for the quantitative determination of lactate in biological fluids.

The use of veratrole has been found the most reliable; a color showing maximum absorption at 5200 Å is formed by the reaction in concentrated sulfuric acid between the ortho dimethoxy-benzene and the oxidation product of lactic acid. Since the veratrole can be used in an alcoholic solution, it is much more convenient than reagents which must be weighed out for each determination. The procedure is outlined as developed for the Evelyn photoelectric colorimeter; any instrument can be used by minor changes in volumes used.

To 3 cc. of high purity H_2SO_4 , previously cooled in an ice bath, 0.5 cc. of the lactate solution, which must be sugar-free and protein-free, is slowly added. The fluids are then thoroughly mixed, adequate cooling being provided. Five minutes in a boiling water bath is followed by cooling in ice water, then in a bath at 0°. To the chilled solution is added exactly 0.10 cc. of 0.125 per cent veratrole in 95 per cent ethyl alcohol. The reagent is thoroughly mixed with the acid and the tubes left 30 minutes at 0° for development of the typical pink color. At the end of this time, 3.5 cc. of concentrated sulfuric acid are mixed with the liquid in each tube to halt the color development and to bring the volume to a readable level in the colorimeter. With this procedure, amounts of lactate ranging from 8 to 35 gamma per 0.5 cc. can be measured with a maximum error of ± 0.5 gamma. The amount of pyruvic acid encountered in normal tissues (1 mgm. per cent) is effectively removed by the calcium-copper precipitation combined with the heating in strong acid. If less lactate than 8 gamma per 0.5 cc. is expected, a 0.050 per cent veratrole solution may be substituted for the 0.125 per cent solution mentioned above and the limit extended to 4 gamma per 0.5 cc.

Experimental obesity. BRODA O. BARNES and ROBERT W. KEETON. Department of Medicine, University of Illinois Medical School, Chicago.

Obesity comes from Latin meaning "Eating itself fat," and the law of conservation of energy demands that in order to gain weight, the intake of calories exceed the expenditure. The appetite seems to be one of the controlling factors and governs the intake. One set of experiments has been carried out on rats in which their appetite was stimulated over a prolonged period by means of protamine insulin. The dosage has run as high as 32 units per kilogram twice daily. The rats must have food avail-

¹ Aided by grants from the Committee for Research in Endocrinology of the National Research Council and the Carnegie Corporation.

able at all times and should it be removed for as short a time as 2 hours, fatal hypoglycemia may be observed. The mortality has been high in such animals and at autopsy the gastro-intestinal tract is empty. The weight gain in adults is very rapid, averaging as much as 3 to 4 grams daily. In the animals surviving for some time the fat depots throughout the body are increased. When compared to animals of similar length, some of these treated animals are as much as 75 per cent overweight. Chemical analysis on the tissues are not yet complete. The reproductive tract is likewise being studied.

A striking syndrome of obesity has developed in a small percentage of dogs in which a total hypophysectomy was attempted. In three animals (two males and one female) the preoperative weight was doubled or trebled within a year after operation. An attempt is being made to analyze the cause of this weight increase as to whether it is an increased consumption of calories, a decreased expenditure, or both. Control animals kept under similar experimental conditions have not shown a similar change in weight or appearance.

Seasonal variations in the epidermal impedance of human skin. A. BARNETT (introduced by S. E. Barrera). Department of Psychiatry, New York State Psychiatric Institute and Hospital, New York City. (Read by title.)

Measurements of the epidermal impedance of the skin of the upper arm by the 3-electrode method at 11,160 cps. (J. Physiol. **93**: 349, 1938) in 20 normal subjects, using an annular electrode having a surface area of 6 cm.², showed substantial constancy of impedance between December and April, inclusive, the values being of the order of 100 ohms (West. J. Surg. **45**: 540, 1937). Between early May and mid-June, depending upon the individual, the impedances begin to rise and attain a maximum value in the course of the summer, varying from 140 to 250 ohms, i.e., 40 per cent to 150 per cent above the winter readings. The impedances generally show a decrease in October and return to the winter levels some time before mid-November. The 20 normals, without exception, showed seasonal changes of this type, the rate of increase and decrease, as well as the total magnitude of the impedance change, presenting, however, marked individual differences.

Coincidentally with the summer increase in impedance there was an increase in the phase angle of the skin varying between 1° and 4°.

Fifty institutionalized psychoneurotics showed seasonal impedance changes in all respects superposable on those found in normals. In a mixed group of fifty institutionalized psychotics, all but three exhibited the seasonal variation. Two of the three, one a manic-depressive, and the other a simple schizophrenic, showed constant impedances throughout the winter and summer seasons, while the third, a Parkinsonian presenting a catatonic picture, showed a complete inversion of the seasonal change, the impedances decreasing to a minimum in July and increasing in the fall months.

Since these 100 mental patients, confined in an institution, were not exposed to extreme seasonal temperature changes, and received ultra-violet treatment thrice weekly throughout the year, it is not certain that either temperature variations or variations in solar radiation, are responsi-

ble for seasonal changes in epidermal impedance. Impedance changes are best explained as representing corresponding variations in epidermal thickness.

Average normal values for epidermal impedance previously published (Proc. Soc. Exper. Biol. and Med. 40: 697, 1939) are valid only for the winter season—December to April, inclusive.

Effects of "sensory" cortical lesions on grasp phenomena in the Macacus rhesus. S. E. BARRERA and B. L. PACELLA (by invitation). Department of Psychiatry, New York State Psychiatric Institute and Hospital, New York City.

In the usual case ablation of the narrow strip of stimuable motor cortex anterior to the central sulcus is associated, in the acute stage, with the following phenomena in the opposite limbs; diminution in spontaneous usage as in movements of prehension, a certain degree of flaccidity, failure to exhibit grasping movements of digits in response to palmar contact with objects such as a stick, and weakness. Such an animal exhibits no tendency to even grasp a bar when an attempt is made to suspend it by the involved forelimb. The forelimb manifests all of these phenomena to a greater degree than the hind limb.

In such a monkey, if as a second operation the contralateral post-central cortex is removed while the above phenomena are clearly manifest, certain changes usually occur in the paretic limbs, more evident in the forelimb. This limb may now be used more actively, although deficiently, in movements of prehension. There may be some increase in muscle tone. Most characteristic and impressive, however, are the changes in the grasping phenomena. If an object such as a stick is now brought into firm palmar contact the fingers may flex about it actively and vigorously, even to a degree permitting free suspension of the animal in the air by that limb for a considerable period of time.

These grasping phenomena appear usually within a few hours following the "sensory" cortical removal and persist very definitely for at least seven days subsequent to operation.

That the grasping is at times definitely "forced" in nature may be seen from the fact that the animal seems unable to release its grasp on an object or bar against resistance for periods varying from 5 to 60 seconds. The disability in the limbs opposite the post-central removal is not comparable to that opposite the motor area removal so far, at least, as grasping phenomena are concerned. No forced element is found in the grasping movements in these limbs.

The ascending glucose renal threshold of the domestic fowl. H. T. BATT (introduced by H. H. Dukes). Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y. (Read by title.)

Examination of the literature shows that the glucose renal threshold of the domestic fowl has received little attention. The high fasting blood sugar level of the fowl (Batt, these Proceedings, 1939) makes the location of this threshold of interest. An attempt has been made to answer this question.

First, normally voided excreta collected from fowls following glucose

ingestion were shown to be free from reducing material. Then, using 51 birds, 180 glucose tolerance tests were run (blood sugar estimations by the micro Folin technique) and any voided excreta tested for reducing power (Benedict's test).

The blood sugar curves obtained were divided into 2 groups: 1, those curves obtained from fowls whose excreta had no reducing power; 2, those from fowls which had at least one excreta sample showing reducing power. A mean blood sugar curve was calculated for each group. The peak of the curve for group 1 was at 260, and that for group 2 at 310 mgm. of sugar per 100 cc. of blood. In 78 per cent of the tolerance curves of group 1, the highest blood sugar value fell below 310 mgm.; in 87 per cent of the curves of group 2, the maximum value was above 260 mgm.

The location of the glucose renal threshold of the domestic fowl would thus seem to lie between 260 and 310 mgm. of glucose per 100 cc. of blood.

The accurate determination of hemoglobin, oxyhemoglobin and carbon monoxide hemoglobin (or myohemoglobin) by means of the dropping mercury electrode. J. PERCY BAUMBERGER, Department of Physiology, Stanford University, Calif. (Demonstration.)

The oxygen content of a small volume (e.g., 20 cu. mm.) of blood may be determined by converting the hemoglobin to acid hematin and measuring the change in oxygen content of the solution by means of the dropping mercury electrode of Heyrovsky (Baumberger: *Symposia on quantitative biology*, VII, 1939). The reaction is carried out in a special electrode vessel in which blood is introduced below a layer of water, acid is displaced from a stopcock by Hg and stirring is accomplished by a glass bead with iron core moved with a magnet.

The oxygen content of the solution surrounding the dropping mercury electrode may be approximately tripled when the hemoglobin of oxygenated blood is converted to hematin in the electrode vessel.

With a fixed potential of $E_h - 0.250$ oxygen is reduced at the dropping mercury electrode and the resulting current is indicated by deflections of a galvanometer. These deflections are a linear function of oxygen tension. With a long light lever an accuracy for the determination of the oxygen content of blood equal to or greater than the Van Slyke method may be attained.

The concentration of hemoglobin may be determined by comparing the deflection where fully saturated blood is used with the deflection with an acid solution having an oxygen content calculable from the Bunsen Solubility Coefficient.

The degree of saturation of hemoglobin with oxygen may be determined by the difference in deflections of the galvanometer when fully and partially saturated bloods are used. Similarly the per cent saturation with carbon monoxide may be determined by difference since this gas does not affect the determination of oxygen by the dropping mercury electrode.

Proper acknowledgment of the assistance of Mrs. Kathleen Bardwell, Dr. Otto H. Müller and Mr. Roderick Parker will be made in the final publication.

Homeostatic changes in hemoglobin, blood volume and basal metabolic rate in rats with sarcoma. J. PERCY BAUMBERGER, Department of Physiology, Stanford University, Calif.

The hemoglobin, erythrocyte count, blood volume, basal metabolic rate and weight changes were followed in a series of rats in which sarcoma implants were growing. A fibro-sarcoma furnished by Dr. L. A. Emge, Stanford Medical School was used. Litter rats without sarcoma were studied as controls. Hemoglobin was determined by the acid hematin technique, blood volume by intravenous injection of T 1824 and electro-photometric measurement of its dilution in serum obtained from cardiac puncture. Basal metabolic rate was determined by the open circuit method using the dropping mercury electrode method for the determination of O_2 .

It was found that hemoglobin, erythrocyte count, blood volume, and basal metabolic rate all gradually decreased as the sarcoma grew. These changes were in such proportion to the increase in weight of the sarcoma and decrease in weight of the normal tissues as to meet the metabolic requirements of the rat. In other words the changes were homeostatic in nature.

Proper acknowledgment of the assistance of Gregory Bard, Kathleen Bardwell, Mervyn Shoor and Charles Wallace will be made in the final publication.

Comparison of estimates of blood volume made by congo red and by carbon monoxide. H. C. BAZETT, F. W. SUNDERMAN, M. E. MAXFIELD (by invitation) and J. C. SCOTT. Department of Physiology and the Pepper Laboratory of the University of Pennsylvania, Philadelphia.

Estimates of plasma volume were made on serum by the method described by Sunderman and Austin. The disappearance of the dye was measured on samples taken at 30, 60 and 90 minutes after injection. Estimates of blood volume were made from these values and hematocrit estimates of the cell/plasma ratio. Estimates of total circulating hemoglobin were made by CO by a modification of the method described by Hartmann (see abstract by Maxfield et al). The carbon monoxide and oxygen mixture was usually inhaled between the injection of congo red and the taking of the blood sample 30 minutes later. This and the initial control blood sample were also utilized for the estimate of CO saturation. The red cells were washed in saline twice (to remove congo red) before being hemolyzed.

Blood volume was estimated from the total hemoglobin divided by the mean hemoglobin concentration of the two samples. The red cell volume was calculated by assuming that all the circulating hemoglobin was contained in cells with the volume to hemoglobin ratio found in the samples examined.

Comparisons thus made simultaneously by the two methods encountered some difficulties, since the discomfort of continued breathing into a spirometer apparently caused some instability. This was reflected not only in slight changes in the hemoglobin concentration and in the hematocrit value but also in distortions of the dye-away curve for the dye. Consequently estimations could be made less accurately. Comparisons made independently by the two methods on successive days had some advantages. The sums of the volumes of plasma and cells thus separately determined were assumed to be the real blood volumes. The estimates made from the dye injection or carbon monoxide inhalation alone were expressed as percentages of these values. The estimates made by congo

red averaged 103.2 ± 2.1 per cent (standard deviations) and those by carbon monoxide 96.2 ± 2.5 per cent of the combined estimates.

We would like to thank the John and Mary R. Markle Foundation whose support made this work possible.

Sphincter and radial iris muscle reaction to high oxygen. JOHN W. BEAN and DAVID F. BOHR (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

The sphincter and radial muscles isolated separately from beef iris were suspended from an optical lever into a continuously oxygenated tyrode bath in a pressure chamber. Compression with oxygen to 70 pounds caused a continuous decrease in sphincter tonus. Decompression to atmospheric pressure induced a recovery. Occasionally the decrease in tonus was preceded by an initial temporary rise which could be eliminated by previous atropinization of the bath. The drop in tonus during the maintenance of the increased pressure, and the recovery on decompression, remained unaffected by such atropinization. Adrenalinization of the bath caused a drop in sphincter tonus, but since the radial muscle showed no contraction on exposure to high oxygen it appears that the loss of sphincter tonus induced by high oxygen is due to a direct action of oxygen on the muscle, rather than to an intermediate release of sympathetico-mimetic substance from intrinsic nerve endings. The radial muscle was less profoundly affected by high oxygen than was the sphincter. That the decrease in sphincter tonus was due to oxygen rather than to pressure itself was demonstrated by the absence of such decrease in control procedures using air. The experimental results indicate that the pupillary dilatation in animals exposed to high oxygen is not necessarily dependent upon either central nervous, or hematogeneous connections. Inherent rhythmicity of sphincter muscle at atmospheric pressure became most prominent after cessation of bubbling the bath and occurred only at relatively low tonic levels. This rhythmicity under some conditions may assume considerable significance in the occurrence of hippus.

Effect of various cholانات on hepatic blood flow and secretion. A. L. BERMAN, F. S. GRODINS and S. L. OSBORNE (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University, Chicago. (Demonstration.)

The methods and results of calibration of the thermostromuhrs used will be demonstrated. The method used for chronic biliary fistula will be shown along with the detailed results of various oxidized and unoxidized bile salts on the output and composition of bile.

*The regulation of bile salt output.*¹ A. L. BERMAN (by invitation), E. SNAPP (by invitation), A. J. ATKINSON (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Approximately 90 per cent of cholates introduced into the intestine can be "recovered" during one enterohepatic circuit. Biliar-duodenal fistula dogs fed our standard diet every eight hours without the return of bile to the intestine produce a relatively constant quantity of cholates, approxi-

¹ This investigation was assisted in part by E. L. Dawes.

mating 0.46 gram per eight hours. If the normal animal synthesizes 0.46 gram per eight hours and loses 10 per cent of the total bile salts returned to the intestine every eight hours, it follows that, regardless of the initial quantity of bile salts in the animal organism, a *balance* should *eventually* be reached when the amount "lost" equals the amount newly formed. This relationship can be expressed in the following equation:

$Q_n = A^n Q + B \left(\frac{A^n - 1}{A - 1} \right)$, where n represents the number of periods; Q_n , the quantity of bile salts excreted at any subsequent period n ; A , the percentage recovery of the total cholates introduced into the intestine, 90 per cent; B , the basal bile salt output on the diet, 0.46 gram; and Q , the initial dose of cholates.

Our experimental results on 5 dogs only approximate the equation when the initial dose of cholates returned is 0.46 gram, or the basal eight hour bile salt output synthesized on the diet alone. In our experiments, all the bile secreted every eight hours is returned to the duodenum, except a small sample for chemical analysis. With larger initial doses, 3 and 6 grams, the equation does not predict the output during the first 2 to 4 days, but does predict the later results since a plateau of cholate output ranging between 4.0 to 5.0 grams per eight hours is reached regardless of the initial dose. It appears that, when a "sudden excess" of bile salts is introduced into the intestine of a dog, the liver, during the first few days either ceases to form new cholates, or more than 10 per cent of the cholates is lost during each enterohepatic circuit, or both. The equation for all possible cases has yet to be derived.

The inactivation of substituted phenylpropyl (sympathomimetic) amines.

KARL H. BEYER (introduced by W. J. Meek). The Department of Physiology, University of Wisconsin, Madison.

As part of an investigation concerning the relation of molecular configuration to mode of inactivation of a group of structurally related "sympathomimetic" amines, experiments have been carried out on a number of substituted phenylpropylamines. The following compounds were studied: *a*, β -phenylisopropylamine (amphetamine); *b*, γ -phenylpropylamine; *c*, α -methyl- γ -phenylpropylamine; *d*, β -phenyl- β -hydroxy-isopropylamine (propadrine); *e*, β -phenyl- β -hydroxy-isopropyl, methylamine (ephedrine); *f*, β (*p*-hydroxyphenyl)-isopropylamine (paredrine). *g*, β (*p*-hydroxyphenyl)- β -hydroxy-isopropyl, methylamine (suprifen); *h*, β (3,4-dihydroxyphenyl)-isopropylamine; *i*, β (3,4-dihydroxyphenyl)- β -hydroxy-isopropylamine (cobefrin).

The enzyme preparations used were an extract of rabbit liver similar to the amine oxidase of Blaschko, Richter and Schlossman (Biochem. J., 1937), and a phenolase similar to the Tyrosinase of Graubard and Nelson (J.B.C., 1935). The Warburg apparatus was used.

Of the amines used, *a*, *c*, *d* and *e* were not inactivated by either enzyme preparation. However, γ -phenylpropylamine, *b*, was oxidized by the amine oxidase preparation but not by the phenolase extract.

Simple amine oxidase extracts did not oxidize the monohydroxy compounds, *f* and *g*, but inactivated the dihydroxyphenylpropylamines at a rate approximating that of autoxidation of *h* and *i*, there being an initial lag in the oxygen uptake.

The phenolase oxidized compounds having 1 or 2 hydroxyl groups on the phenyl ring (*f*, *g*, *h* and *i*) but did not oxidize any of the others. Oxygen uptake in the presence of monohydroxy was much greater than in the presence of dihydroxy derivatives, but the character of the corresponding curves (oxygen uptake with respect to time) for the two monohydroxyphenyl compounds differed considerably from each other. Paredrine, *f*, was very rapidly oxidized while suprifin, *g*, was oxidized to the same extent only after a greater period of time. Solutions of phenolase buffered at pH 6.36 and pH 8.00 showed less oxygen uptake in the presence of the hydroxyphenylpropylamines than did those buffered at pH 7.3.

This work indicates that while some of the phenylpropylamine derivatives are relatively stable in biological media, those having a terminal amine group on the side chain or one or two hydroxyl groups on the phenyl ring are capable of undergoing oxidative inactivation.

Sulfanilamide and increased sensitivity to light. H. F. BLUM. The National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

Clinically, sulfanilamide is known to render patients hypersensitive to sunlight. The mechanism has been variously explained. Recently, Epstein (*J. Investigative Dermatol.* 2: 43, 1939) has shown that skin may be sensitized to mercury arc radiation, locally, by intradermal injection of sulfanilamide.

The present experiments show the following:

I. The sulfanilamide response is not dependent upon O_2 , thus indicating its essential difference from photodynamic action. By depriving the skin of O_2 , the latter is inhibited, but not the former.

II. The sulfanilamide response resembles normal sunburn morphologically and in its time relationships; erythema appears after a latent period, persists some days and is followed by pigmentation. It differs in both respects from photodynamic action which is characterized by immediate and transitory whealing.

III. The response occurs only when sulfanilamide is injected previous to irradiation.

IV. The active wave-lengths cannot be distinguished, by tests with filters, from those which produce normal erythema and pigmentation. These are not the wave-lengths to which porphyrins photosensitive.

V. The sulfanilamide response appears earlier, and runs a more severe course than the accompanying normal erythema and pigmentation of the surrounding area. Both processes are affected proportionately by varying doses of radiation. The same parallelism is observed when wave-lengths are varied.

Sulfanilamide is not a photodynamic sensitizer, and it is unlikely that it acts to produce substances which serve as such sensitizers (e.g., porphyrin has been proposed).

Two possibilities remain; 1, sulfanilamide acts as the light absorber in a photochemical reaction different from that underlying photodynamic action, and 2, sulfanilamide renders skin tissue (presumably the prickle cells) hypersensitive to the action of normal erythema producing ultra-violet radiation. The latter seems the more probable explanation.

The relation of oxygen consumption and environmental temperature to the growth of dwarf mice. EDWARD G. BOETTIGER (introduced by W. R.

Amberson). Department of Physiology, University of Maryland Medical School, Baltimore.

A wide variation in growth prevents an accurate study of pituitary dwarf mice. This variability is apparently due in large part to the difference in the lengths of critical periods which begin about the 17th day of age and are characterized by a loss of body weight. A study of these periods was made in an effort to produce more uniform animals. The basal oxygen consumption (87 determinations) was measured at 33°C. on dwarf mice at various ages and compared with a small number of determinations on young normal mice. A striking relationship between the rate of growth and the rate of oxygen consumption was evident. The 16 per cent average decrease in weight of critical period dwarf mice was paralleled by a 17 per cent drop in oxygen consumption. A return to the pre-critical level occurred when body weight increased. The youngest dwarf and normal mice had identical rates of oxygen consumption per unit surface but while the normal's rate doubled during the second week that of the dwarf remained low. Thus in older animals the metabolism of the dwarf is 40 per cent below that of the normal mouse.

Dwarf mice are unable to maintain body temperature during the critical period. Isolation at this time results in a very reduced body temperature and death. Dinitrophenol returns the temperature to the normal range. Pre-critical dwarfs isolated at 34°C. do not lose weight but continue to grow at a rate characteristic of the post-critical dwarf mouse. The ability to control body temperature at an external temperature of 15°C. develops only in the older dwarf mice. A high environmental temperature allows the expression of an inherent growth capacity. The close relation between oxygen consumption and growth rate may be, at least during the critical period, the result of the inability to maintain body temperature.

A new type of permanent pancreatic fistula (permitting the regulation of the outflow of the secretion). W. N. BOLDYREFF and O. E. THOMPSON (introduced by W. R. Bloor). Battle Creek Sanitarium, Battle Creek, Mich.

The classical Heidenhain-Pavlov operation for the pancreatic fistula has the handicap that through it large amounts of pancreatic juice are lost. This renders the animals pathologic even in the first days after the operation ("pancreatic triad," W. N. Boldyreff) and they sometimes die from this cause two or three weeks after the operation. All such animals are doomed and die within a year.

The method of Ivy and also that of Boldyreff and Martin do not have the defects mentioned but the animals subjected to these operations cannot be considered normal; to keep the animals normal is the main objective of the operation described.

It consists of combining the usual duodenal fistula made in the proper place with a special kind of pancreatic fistula. The first part of this operation is well known and requires no description. The second part consists of insertion of a glass cannula into the main (or small) pancreatic duct from the inside of the intestine through the natural orifice. This glass cannula is kept in place by a strong silk or linen ligature which is tightly tied on its neck on the outside of the pancreatic duct. A rubber tube, about eight inches long, is put on the free end of the glass cannula. A

string is attached to the end of this rubber tube allowing it to be pulled outside through the metallic tube of the duodenal fistula.

When no experiments are conducted the rubber tube lies in the intestine; the juice flows freely into the intestine, takes part in digestion and is then absorbed. During the experiment the end of the rubber tube is drawn outside and one can collect pure juice from it. The rubber and the string are then concealed inside the fistular duodenal tube. The end of the string is hanging outside and is covered with a special cap which fits into the rim of the duodenal tube.

Studies on the physiology of respiration at high altitudes. WALTER M. BOOTHBY, W. RANDOLPH LOVELACE, II (by invitation) and OTIS O. BENSON, JR.¹ (by invitation). Rochester, Minn.

Curves will be shown illustrating the alveolar oxygen and carbon dioxide pressure at various altitudes when breathing air. Quantity of oxygen necessary to maintain an essentially normal alveolar oxygen pressure has been determined for elevations up to 35,000 feet. Rapidity of ascent and danger of aeroembolism will be discussed with especial reference to preliminary nitrogen decompression by inhalation of 100 per cent oxygen. New type of sponge rubber expiratory-inspiratory valve for use on the oxygen inhalation apparatus will be demonstrated. This valve remains efficient at very low temperatures. A convenient valve for obtaining alveolar air by the Haldane method will be shown.

The cortical taste area in monkeys and a semi-quantitative method of testing taste in monkeys. WALTER S. BÖRNSTEIN (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

The cortical taste area in man from clinical studies is generally believed to be localized either in the temporal lobe or in the insula. Other clinical investigations (Börnstein, Mschr. Psychiat. Neurol. 67:216, 1928) indicate that in man the cortical taste area is located in the parietal operculum, at the foot of the postcentral gyrus and hence adjacent to the somato-sensory and motor areas for the tongue and to the masticatory area. To elucidate these observations and to permit a more precise localization, the gustatory acuity of monkeys has been tested by the "preference method" preparatory to cortical ablations. Solutions representing each of the four taste qualities were offered singly, paired with water, and the concentrations were reduced until the animal failed to demonstrate a preference. Bilateral ablation of the sensory face area effected in one monkey a marked gustatory impairment, but only for five days. Additional bilateral ablation of the motor face area produced severe gustatory deterioration. In another monkey the two first operations, i.e., ablation of one sensory face area, ablation of the other sensory and one motor face area, had no decisive effect on the gustatory function. Additional removal of the second motor face area produced a severe gustatory impairment. After several weeks, the length of time varying for the different taste qualities, recovery of the gustatory function commenced. These preliminary experiments suggest that the taste area in monkeys coincides with the sensory and motor face areas.

¹ Captain, Medical Corps, U. S. Army.

The mechanism of augmented pulmonary ventilation resulting from intermittent central stimulation of the vagus. T. E. BOYD. Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Ill.

In vagotomized dogs, weak central stimulation of one vagus, so timed as to cut short each inspiration, also accelerates respiratory rhythm. Hillenbrand and Boyd (Am. J. Physiol. 116: 380, 1936) suggest that acceleration results because the center is not allowed to discharge completely, being thus an indirect consequence of the periodic inhibition. Hammouda and Wilson (J. Physiol. 94: 497, 1939) find that the minute ventilation is increased. They argue therefrom that the acceleration is due to a separate afferent mechanism, with a positive excitatory effect upon the center.

The latter interpretation implies that the vagal action increases the total number of impulses discharged per minute from the inspiratory center, and assumes that ventilation rate is a reliable index of this discharge. The assumption is unsafe for several reasons, among them the fact that in the typical long-drawn-out inspiration of the vagotomized animal much energy is wasted in maintaining lung volume without adding to ventilation. The time factor may be taken into account by using, as an index of central activity, the area of each inspiratory curve (change of lung volume, or of intrapleural pressure) multiplied by the frequency.

Simple rebreathing, in the vagotomized dog, augments minute ventilation and the minute area of the inspiratory curves in nearly the same ratio. In this condition excitation is manifest by increased depth and frequency, the duration of inspiration remaining constant. Periodic vagal stimulation, in the manner described above, reduces inspiratory duration as well as depth. The ventilation is increased, provided the inspirations are not cut short too early. But the minute area of the inspiratory curves is reduced. Augmented ventilation is therefore probably not due to central excitation, but to more effective grouping of the impulses discharged.

The excitability of visceral muscles during inhibition. EMIL BOZLER. Department of Physiology, The Ohio State University, Columbus. (Read by title.)

Non-pregnant cats which had received injections of theelin for a few days were used in experiments on the uterus. The electric excitability of the organ was tested by stimulating it directly with single condenser discharges. During inhibition produced by stimulating the hypogastric nerve or by injecting adrenaline the muscle became completely non-excitable. In the rabbit's gut, adrenaline in moderate amounts did not slow the frequency of the rhythmic contractions, but shortened the duration of the discharge of impulses during each contraction. Adrenaline also decreased the electric excitability of intestinal strips. The experiments suggest that inhibition of visceral muscles is due to a depression of the conduction of muscular impulses resulting from the diminution in excitability.

Observations following the cessation of treatment of the infantile female rat with androgens and gonadotropic extracts. JAMES T. BRADBURY (introduced by A. E. Woodward). University of Michigan Medical School, Ann Arbor.

Forty-nine female rats were given 0.3 mgm. of testosterone propionate (Neohombreol) on alternate days from the 6th until about the 30th day of age. Vaginal patency occurred at 14 to 20 days of age in 35 of these rats. The formation of the lower vagina was inhibited in the others. Vaginal smears were diestric during treatment and continued so until about 50 days of age. By the 60th day all the rats with a patent vagina exhibited a continuous vaginal estrus which has persisted in rats observed for a year.

Thirty-two rats were given 5 to 10 rat units of pregnancy urine APL (Antuitrin-S) daily from the 6th until the 30th and 50th days of age. Definite masculinization was produced by this treatment. Vaginal patency usually occurred from the 14th to the 20th day of age. Estrous smears were continuous from the time of vaginal patency until the cessation of treatment. After cessation of treatment this vaginal estrous continued in a high percentage of the rats observed. Similar results were obtained in 16 rats treated daily with 0.5 rat units of pregnant mare's serum (Gonadogen).

The prolonged estrous phase following cessation of treatment has given rise to several pathologic conditions. There has been a high incidence of large abscesses originating in the oviduct and periovarial capsule. These abscesses usually destroyed the ovary. A massive hypertrophy of the vagina and cervix has occurred in several of the rats. Mating attempts indicate the rats have been rendered permanently sterile.

A comparison of the effects of desoxycorticosterone and cortico-adrenal extract on adrenal insufficiency. S. W. BRITTON and E. L. COREY. Physiological Laboratory, University of Virginia Medical School, University. (Read by title.)

Observations have been brought forward by different workers in the past year which indicate that desoxycorticosterone shows no significant influence on carbohydrate metabolism in cases of adrenal insufficiency. This seemed very singular, since the material very effectively alleviates the symptoms of adrenal loss or hypofunction, and to do so one would expect to observe an effect in the carbohydrate levels in keeping with the action of cortico-adrenal extract.

Comparison has been made of the influence of desoxycorticosterone acetate ("Cortate," synthetic preparation, Schering Corporation) and corticoadrenal extract on the general symptoms of adrenal insufficiency, the various carbohydrate levels, and the blood serum electrolytes. Several series of cats have been used.

Desoxycorticosterone did not alleviate the symptoms of adrenal insufficiency in the cat nearly as quickly as cortico-adrenal extract. In some cases when animals were prostrated it failed to bring about restoration, even when used in large doses.

In the long-surviving adrenalectomized animal, desoxycorticosterone maintained high levels (normal or supernormal) of blood sugar and liver and muscle glycogen, and also normal levels of serum potassium, sodium and chloride. In these respects it acted equally as well as cortico-adrenal extract.

In adrenalectomized cats showing severe symptoms of adrenal insufficiency, desoxycorticosterone raised the subnormal blood sugar values to normal in six or eight hours, and at the same time increased the liver and

muscle glycogen levels slightly. The effects on carbohydrates were not nearly as marked as those brought about by cortico-adrenal extract. Serum electrolytes were correlatively restored to normal by desoxycorticosterone.

Further observations on the glycolytic function of the adrenal cortex. S. W. BRITTON and R. F. KLINE (by invitation). Physiological Laboratory, University of Virginia Medical School, University. (Read by title.)

Cortico-adrenal extract appears under a number of different experimental conditions to be a much more effective glycogenetic agent than insulin.

In the presence of large amounts of glucose, adrenaless cats are able to raise their liver, skeletal muscle and cardiac glycogen levels scarcely at all, and insulin provides no glycogenetic aid. Cortico-adrenal extract rapidly alleviates all the carbohydrate deficiencies, however, and even in the absence of injected glucose produces large increments in liver and cardiac glycogen values.

In the case of pancreatectomized-adrenalectomized cats, death occurs particularly and firstly because of cortico-adrenal loss. Hypoglycemia and liver and cardiac glycogen exhaustion are the usual conditions observed. The pancreatectomized-adrenalectomized animal, dying with severe symptoms, may be restored time after time by cortico-adrenal extract administration alone. Blood sugar elevation is a significant feature in such cases.

Further studies on the passage of anions into the central nervous system.

B. B. BRODIE (by invitation) and G. B. WALLACE. Department of Pharmacology, New York University College of Medicine.

We have shown that the extracellular fluid of the central nervous system forms a distinct fluid compartment with a barrier preventing the free passage of various ions into it from the blood. For iodide and thiocyanate, a serum threshold concentration exists below which the anions fail to enter the central nervous system.

The three sets of experiments reported here were performed on dogs. When bromide or iodide was injected intravenously, analyses showed that the ratio of the administered ions to chloride, that is the chloride replacement, was constant in all parts of the central nervous system and cerebrospinal fluid, showing that these ions were evenly distributed throughout the central nervous system extracellular fluid.

When the ions were injected intracisternally the ratio of the administered ions to chloride was uneven, the greatest replacement being in those parts adjacent to the cistern, the replacement in the cortex and lumbar cord being negligible.

In the third set, the spinal cord and membranes were ligated in the cervical region, thus shutting off all connection from above. Bromide was injected intravenously and analyses showed the bromide to be in the spinal fluid and cord in amounts comparable to those in the cisternal fluid.

If the choroid plexus were the only place of entrance for the ions, then only active circulation of the cerebrospinal fluid would account for the even distribution throughout the central nervous system following intravenous injection. That such a circulation does not exist is shown by the lack of even distribution after cisternal injection. We conclude then,

that whatever part the choroid secretion may play in the passage of the ions into the cerebrospinal fluid, these leave the blood vessels and enter the fluid compartment of the central nervous system throughout its entire extent. This passage differs from that in other organ tissues in that there are factors which influence it either through a selective permeability or filtration-reabsorption mechanism.

Post-exercise orthostatic collapse. ELIZABETH BROGDON (by invitation) and F. A. HELLEBRANDT. Department of Physiology, University of Wisconsin, Madison.

In 60 experiments on 3 healthy young women the cardiovascular response to standing was studied under circumstances which varied the physiological state of the anti-gravity muscles. Thirty minutes of verticality were tolerated consistently when the subjects stood relaxed without inhibiting postural sway. The systolic pressure remained near the stabilized sitting value. The diastolic pressure rose slightly. The heart rate, usually 10 beats or less above the sitting level, showed rhythmic fluctuations.

When immobilized, one subject after 20 minutes of standing, twice exhibited a rapid rise in heart rate, a sudden drop in systolic pressure and syncope. In general, the response to fixed standing was marked by a progressive but gradual rise in heart rate, a falling pulse pressure as the diastolic elevated, and a relatively constant systolic.

Two of the subjects were usually at the point of collapse in 15 minutes or less when brief violent exercise on the electro-dynamic brake bicycle ergometer preceded the fixed standing. Heart rates rapidly fell to approximately 115 per minute from exercise values near 200 and *then began to rise*, approaching the immediate post-exercise level. The diastolic pressure rose and the systolic fell below the stabilized figure, resulting in very low pulse pressures. The third subject demonstrated a similar but much less marked trend in heart rate, an adequate blood pressure throughout the post-exercise period and no tendency to collapse.

When the post-exercise stance was relaxed and associated with normal postural sway, the delayed rise in heart rate was less frequently manifest. Few cases of collapse occurred. When they did appear, it was late in the standing period.

The collapse observed is similar to the "gravity shock" of sport participants described by Mateef (Ztschr. f. d. ges. exper. Med. **85**: 115, 1932) and the orthostatic syndrome produced in normal subjects upon the administration of sodium nitrite by Weiss, Wilkins and Haynes (J. Clin. Investigation **16**: 73, 1937). Our experiments emphasize the importance of postural sway in maintaining an adequate circulating blood volume when the hydrostatic handicap of the erect position is aggravated by local vascular dilatation induced in the lower extremities through exercise.

*A study of the effect of estrin on the responses to genital stimulation shown by decapitate and decerebrate female cats.*¹ R. B. BROMLEY (by invitation) and PHILIP BARD. Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md. (Read by title.)

¹ Aided by grants from the Committee for Research in Problems of Sex, National Research Council.

In decapitate female cats unilateral application of a light intermittent mechanical stimulus to the vulval or perineal region causes *a*, deflection of the tail to the opposite side; *b*, turning of pelvis toward the stimulated side; *c*, ipsilateral flexion and contralateral extension of the hindlegs, and *d*, alternate movements of the hindlegs in the asymmetrical attitude established by *c*. Bilateral stimulation evokes some dorsiflexion of the pelvis and alternate hindleg movements; with the preparation in the prone position this response resembles the treading characteristic of normal females in estrus. But none of these responses depends on a state of estrus; all are readily obtained in ovariectomized females decapitated when completely anestrous, and they can be evoked in decapitate males.

In cats decerebrated at a pontile or lower mesencephalic level vulval stimulation gives essentially the same responses as in decapitate preparations, but alternate or rhythmic movements are rarely elicited. Again, these do not depend on estrus. All decerebrate females, whether wholly anestrous or fully in heat, regularly show a complete collapse of the extensor rigidity of the forelegs and a moderate relaxation of that of the hindlegs when the distal vagina is gently stimulated mechanically. This response is specifically vaginal in origin; it cannot be obtained by stimulation of anus, rectum, vulva, urogenital sinus or neck of bladder, and no trace of it could be evoked in twelve decerebrate male cats by any kind of genital stimulation. The inhibition of the foreleg extensors is tonic; during application of the stimulus the shortening reaction is absent. The response imposes on the standing decerebrate animal an attitude which suggests the estrous crouch of normal cats. It may denote a bulbospinal mechanism concerned with the major postural adjustment of feline estrus. The reaction cannot be elicited in decerebrate bitches, a fact of possible significance since crouching is not a part of canine estrous activities.

It is concluded that spinal and decerebrate female cats in a state of estrus show no reactions suggestive of normal sexual behavior which are not evocable in similar anestrous preparations.

Rôle of neocortex in the control of certain postural responses of the opossum (Didelphys virginiana). REG. B. BROMLEY (by invitation) and CHANDLER McC. BROOKS. Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md. (Read by title.)

Electrical stimulation of the cerebral cortex of 10 young and 7 adult opossums revealed clearly localized points from which respiratory acceleration, rotation of the contralateral ear, fore-leg movements and flexion of the opposite hind-leg could be obtained. Other responses were elicited with less regularity.

The opossum shows hopping and placing responses which are similar to those of other mammals. These reactions are, however, somewhat slower and are less easily evoked than those of higher forms. Like rats and monkeys, opossums tend to rotate their feet and grasp objects with which they come into contact. This habit interferes with the elicitation of the hopping reactions and certain placing responses.

Ablation of the electrically excitable fore- and hind-leg areas of the cortex of one hemisphere resulted in a loss of placing responses and a marked deficiency in the hopping reactions of the contralateral legs. Removal of the entire neocortex produced no greater deficiencies in postural reactions

than did ablation of fore- and hind-leg areas alone. Bilateral lesions produced bilateral deficiencies. Lesions in non-excitabile regions of the cortex caused no deficiencies in these responses. Though the cortex of this marsupial is not essential to many types of reaction which in higher forms are abolished by decortication it, nevertheless, plays a rôle in certain postural adjustments.

In the opossum as in the rabbit a hind-leg cortical representation is not easily demonstrated but in 11 of the 17 animals stimulated discrete hind-leg movements were obtained. Hind-leg flexion was more readily evoked in young animals than in adults. The existence of a cortical control of hind-leg reactions was likewise demonstrated by the fact that ablation of the electrically excitable cortical areas abolished the hind-leg placing responses and rendered the hopping reactions definitely subnormal.

Neural factors determining the frequency of impulses discharged from a ganglion cell. D. W. BRONK and M. G. LARRABEE (by invitation). Johnson Foundation, University of Pennsylvania, Philadelphia.

Under experimental conditions a nerve cell in a sympathetic ganglion can be excited by synchronous volleys of impulses in the several preganglionic fibers which converge upon the cell. There is then one impulse discharged for each incoming volley, provided the frequency of stimulation is not above about 40 per second. Naturally, however, the preganglionic fibers conduct from the sympathetic centers trains of impulses which are of different frequencies. Accordingly there can be no simple relation between the frequency of impulses in the preganglionic fibers and the rate of discharge from a ganglion cell.

In order to investigate the factors which determine the frequency of action in a nerve cell we have isolated single fibers in the postganglionic nerve from the stellate ganglion. While thus recording the action potentials from a single cell we have stimulated varying numbers of preganglionic fibers at various frequencies. In this way we have shown that the frequency of discharge from a cell is governed both by the frequency of impulses in the individual fibers and by the number of fibers bringing impulses. If the frequency of excitation, measured in terms of the total number of impulses arriving in a unit of time, be sufficiently great, the cell may continue to discharge for some seconds after the end of stimulation.

The frequency of cellular response to a certain rate of incident impulses varies widely from cell to cell, it is modified by the chemical environment of the cell and is depressed for some time following a period of previous activity.

By thus observing the response of a single nerve cell to the asynchronous activity in the many fibers to which the cell is subject it is possible to analyze more clearly the processes involved in the development of excitation within the nervous system by the normally occurring trains of impulses.

A study of the mechanism whereby copper acetate and certain drugs produce ovulation in the rabbit. CHANDLER McC. BROOKS, WILLIAM G. BEADENKOPF (by invitation), and SAMUEL BOJAR (by invitation). Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md.

Intravenous injection of 10 to 15 mgm. of copper acetate produced ovulation in normal rabbits (confirming Fevold, Hisaw and Greep. This Journal 117: 68, 1936). Animals anesthetized with nembutal at the time of injection likewise ovulated. Eight rabbits in which the hypophyseal stalk had been transected one to six months previously failed to ovulate following the injection of 10 to 20 mgm. of copper acetate. This copper salt was ineffective even when given within 15 to 30 minutes after stalk transection (6 animals). The hypophyseal stalk was severed in six normal mature rabbits within 30 to 90 minutes after 15 mgm. of copper acetate had been administered; none ovulated. In eight additional cases stalk section was performed 110 to 300 minutes after injection of the copper salt. Six rabbits ovulated indicating that sufficient time had elapsed between injection and the operation for the material to exert its effect.

Marshall (*Les Hormones Sexuelles*, Singer-Polignac Colloque, Paris, 1938) reported that picrotoxin induces ovulation in the rabbit. We found that 1.0 to 1.5 mgm. of drug produced ovulation in 5 of the 18 normal rabbits injected. Eight chronic stalk-cut animals and 3 in which the stalk had been transected 2 to 3 hours before injection did not ovulate when corresponding amounts were administered though equally intense convulsions resulted. Metrazol (100 mgm. per kgm., subcutaneously) produced ovulation in 4 out of 14 cases. Three stalk-cut rabbits failed to ovulate when given identical amounts of metrazol but this can not be considered significant since so few normal animals ovulated.

Copper acetate, picrotoxin and metrazol can excite an ovulatory response in rabbits. The fact that the response to copper acetate and picrotoxin is abolished by transection of the hypophyseal stalk before or shortly after injection suggests a neural stimulation of the hypophysis resulting from some central excitatory action of these materials.

The initial contraction process. DUGALD BROWN and F. J. M. SICHEL.
New York University and The University of Vermont.

As shown previously by the method of abrupt compression and decompression, the potential energy in the twitch is mobilized in its entirety during the initial tenth of the contraction phase and thereafter disappears from the system. The apparent association of this initial mobilization process with the initial state of excitation led us to examine the effect of electrical stimuli interposed during its course. For this purpose silver-silver chloride electrodes (2.5 x 4 cm.) placed parallel to the muscle fibers were used. In this way all parts of the muscle were excited simultaneously, thus obviating dependence on a propagated disturbance as the exciting agent. The isometric tension was used as an index of the effectiveness of stimulation.

The results show that the second of a pair of identical rectangular shocks of 0.4 msec. duration may augment the action of the first during a brief period of about 5.0 msec., a maximum effect being attained in about 3.5 msec. This phenomenon precedes the usual summated responses resulting from stimuli interposed later in the contraction cycle.

The results of applying single shocks of variable duration show that at constant voltage a maximum response is obtained at 4 to 5 msec., currents of longer duration causing a reduction in tension.

In general the results indicate the existence of an initial process, a type

of local reaction, whose magnitude conditions the tension subsequently developed. At 18°C. the process terminates in 3 to 4 msec., independently of a stimulating current which may persist for a longer period of time.

The effect of prolonged stimulation on the relative refractory period in nerve.

E. T. VON BRÜCKE (by invitation), A. FORBES and M. EARLY (by invitation). Department of Physiology, Harvard Medical School, Boston, Mass.

Recording from a frog's sciatic nerve with a cathode ray oscillograph, we studied the relative refractory period by delivering two induction shocks at varying intervals with a Lucas pendulum. Fatiguing series of maximal stimuli were delivered through electrodes, proximal to those used for the test shocks, at a frequency of 120 per sec. for durations varying from 3 to 60 sec.

The first of the pair of testing stimuli was always supramaximal; the second was sometimes supramaximal, for the study of size of response, sometimes submaximal, for the study of changes in excitability.

Immediately after cessation of rapid stimulation for 30 to 60 sec. the size of response to either maximal or submaximal second stimulus was reduced during the early part of the relative refractory period, no reduction appearing in the response to the first stimulus. As Graham and Lorente de N6 (Am. J. Physiol., 1938) found the recovery of size of response more rapid than that of excitability, so we find the recovery of size of response less affected by prolonged activity than the recovery of excitability. Slight, transient depression of the maximal response is found early in the relative refractory period; subsequent tests at 5 or 6 sec. intervals show gradual return to the value preceding "fatigue." With a submaximal second stimulus the depression due to prolonged activity is much greater, persists longer, and is found in later stages of the relative refractory period. This signifies raised threshold reducing the percentage of fibers responding, and is evidence of increased impairment of excitability in the relative refractory period, due to "fatigue."

The results were almost identical with test stimuli applied 40 to 80 msec. after cessation of the fatiguing series; "fatigue" is therefore many times more enduring than the relative refractory period.

A fatiguing series of 3 to 5 sec. caused similar but smaller and much more transient effects.

Although, with the stimulus frequency used, we found no reduction in size of response to single stimuli after long stimulation (cf. Forbes and Rice, Am. J. Physiol., 1929), we found a prolonged decrease in excitability to a single stimulus.

The heat production of a non-heat regulating dog at various temperatures.

JOHN M. BRUHN, University of Alabama.

The heat production of a non-heat regulating dog (pontile animal) has been studied at rectal temperatures from 30.5 to 38°C.

When food is withheld for 24 hours the body temperature remains at a level of 6°C. above that of the environment. The animal was maintained at the desired temperature for several hours before the metabolism runs.

In the absence of stimulation for an appropriate time the dog is com-

pletely flaccid, but when stimulated stands and walks if confined to a narrow runway. The tests were started the 5th week after operation and continued weekly thereafter.

The heat production when plotted against the rectal temperature is a straight line, there being a 12 per cent increase in metabolism for each degree rise in temperature.

The percentage increase of metabolism per degree rise of temperature from 30.5 to 38°C. appears to be of the same order of magnitude as that of the human subject with fever.

The metabolism of this dog is much higher than that of cold blooded animals at equal temperatures.

*The arterial blood pressure in auricular fibrillation, measured directly.*¹

WILLIAM C. BUCHBINDER and HAROLD SUGARMAN (introduced by L. N. Katz). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Blood pressure determinations on patients with auricular fibrillation made by the ordinary auscultatory method are not only difficult to obtain but are subject to considerable error. Many clinicians rely upon this method, however, in furnishing them with a rough estimation of what the blood pressure may be in this arrhythmia. A smaller number of them omit the procedure, regarding it as an almost worthless one. It was therefore thought desirable to study this problem by actually determining the blood pressure by intra-arterial puncture.

The needle manometer recently devised by Hamilton was used for this purpose. By lining it up with the electrocardiograph machine a simultaneous record of the electrocardiogram and the pulse curve was obtained. The needle was inserted in the brachial artery. Records so obtained from 10 patients furnished the basis of this study; 8 had chronic auricular fibrillation and 1 had chronic auricular flutter, and another sinus rhythm with pulsus trigeminus. In addition to measuring the systolic, diastolic and pulse pressures, the length of the electrical cycle (R-R), the duration of ejection, and the time differences between the electrical and mechanical events (Q-E), were also noted. In each record 50 consecutive beats were so analyzed.

The following are the more significant of the results obtained:

1. Fluctuations of the blood pressures occur from beat to beat in auricular fibrillation. The systolic and pulse pressures show a direct relationship, and the diastolic pressure an inverse relationship to the preceding cycle length.
2. In auricular fibrillation and flutter with regular ventricular rhythm the blood pressure values are nearly constant.
3. The blood pressure fluctuations are dependent on irregular action of the ventricles.

Pepsin performance of the vagotomized entire stomach pouch to histamine.

GLADYS R. BUCHER (introduced by John Gray). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Gastric juice was collected in 3 types of experiments which differed with

¹ Aided by the A. D. Nast Fund for Cardiac Research.

respect to histamine administration and analyzed for pepsin by a hemoglobin method. In series A, 1 mgm. of histamine dihydrochloride was given subcutaneously and the resulting secretion collected at 15-minute intervals for one hour, after which the initial dose of histamine was repeated and collections continued for another hour. It was found that pepsin concentration was maximum at 15 minutes and fell to a minimum at 30 to 40 minutes after the histamine injection, when the volume-rate was maximal. This was followed by a terminal rise at the end of the hour. The response in the second hour was similar to that in the first. The pepsin output was at once increased and attained a maximum at about 30 minutes. This response was found repeated during the second hour. A statistical comparison of hourly pepsin outputs during the first and second hours revealed no significant degree of difference. Collections made over 2 one-hour periods prior to the first histamine stimulation yielded uniformly low outputs of pepsin which cannot be regarded as the true basal rate of secretion, as the acidity of these samples was so low as to permanently inactivate most of the pepsin.

In series B, a uniform rate of secretion was obtained by giving small constant doses of histamine subcutaneously every 10 minutes over 5 to 8 hour periods. Under these conditions the hourly output of pepsin was remarkably constant. In series C, histamine was given as in series B during 3 consecutive periods of 160 minutes each. In the first and third periods, the volume rate per 20 minutes was 13 to 17 cc., while during the middle period the volume rate was augmented to 27 or 30 cc. per 20 minutes by increasing the 10 minute dose of histamine. The result of this series of experiments showed that histamine produces a significant and prolonged increase in the total output of pepsin.

Occurrence of the gonadotropic antagonist and its effectiveness by various methods of administration. CARL A. BUNDE (by invitation) and ARTHUR A. HELLBAUM. Department of Physiology, University of Oklahoma School of Medicine, Oklahoma City.

The gonadotropic antagonist was found in pituitaries of all animals tested (9 species of mammals), but could not be demonstrated in gonadotropic preparations from nonpituitary sources. The antagonist from any one species effectively inhibited concurrent injections of gonadotropic preparations from the same or other species, whether these preparations were of pituitary or nonpituitary sources. Furthermore, antagonism could be demonstrated when tested in the same species from which the antagonist had been prepared.

The antagonist action of pituitary extracts was first demonstrated by intraperitoneal injections and this method has been generally adopted. The authors investigated the possibility of using other routes of administration and found that intravenous injections were strikingly effective, subcutaneous injections produced no results, and it was difficult to demonstrate any effect by means of intramuscular injections. Oral administration was ineffective, as was to be expected, since the antagonist was found to be destroyed by trypsin digestion. It was also found that intraperitoneal injections of antagonist preceding administration of a gonad stimulating principle were effective, while antagonist following administration of a gonadotropic hormone had no effect.

Further study on the electrical potential of the cerebral cortex in relation to consciousness, unconsciousness, and anesthesia. W. E. BURGE, E. G. KOONS (by invitation) and E. L. BURGE (by invitation). Department of Physiology, University of Illinois, Urbana, and Randolph Field, San Antonio, Texas.

The anterior and posterior roots of the spinal nerves in the lumbar region of the cord of etherized dogs were carefully exposed. When an electrode was placed on each of these roots of a deeply anesthetized dog and a galvanometer connected in the circuit a current of 0.09 micro-ampere flowed from the posterior to the anterior root, thus showing the anterior root to be electro-negative in deep anesthesia. As the dog came from under the ether the strength of this current gradually decreased to zero. With further recovery there was a reversal in polarity, and when the dog was only slightly anesthetized a current of 0.06 micro-ampere flowed from the anterior to the posterior root, thus showing the posterior root to be electro-negative in the semi-conscious dog. Upon the death of the animal the potential difference between the two roots promptly disappeared.

The electro-negativity of the anterior roots of deeply anesthetized dogs is interpreted to indicate that more negative charges, or nerve impulses, were leaving the brain by the motor anterior roots than coming to the brain by the sensory posterior roots, resulting in a loss of negative charges, thereby causing the cerebral cortex to become electro-positive in deep anesthesia (Burge, Neild, Wickwire, Orth: *Anesthesia and Analgesia*, March-April, 1936).

The electro-negativity of the posterior roots of lightly anesthetized, semi-conscious dogs is interpreted to indicate that more negative charges were coming to the brain by the sensory posterior roots than leaving by the motor anterior roots, resulting in a gain of negative charges, thereby causing the cerebral cortex to become electro-negative in the conscious state (Neild, Elhardt, Wickwire, Orth, Burge: *Am. J. Physiol.*, June-August, 1936).

So long as an animal is alive negative charges come to the brain by sensory nerves and leave the brain by motor nerves, and upon death these charges disappear. If the outgoing negative charges are in excess, as occurs during anesthetization, the cerebral cortex loses charges and becomes electro-positive with resulting unconsciousness and anesthesia. If the incoming negative charges are in excess the cerebral cortex gains charges and becomes electro-negative with resulting consciousness.

The andromimetic function of the immature rat adrenal. M. W. BURRILL and R. R. GREENE (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Price (1936) showed that after early castration of the male rat the ventral prostate is maintained in a nearly normal functional state for a certain period. Howard (1937) found the same phenomenon in mice and also confirmed Price's findings in the rat. This maintenance of the prostate in the absence of the gonads indicated some extra-gonadal source of androgens. The present authors (1939) offered direct evidence that this source is the adrenal. Castration at 16 days followed by adrenalectomy at 21 days invariably resulted in complete prostatic atrophy at 26 days, whereas

castration alone did not produce complete atrophy. Gersh and Grollman, however, concluded from similar experiments that the adrenal is not responsible for prostatic maintenance in the castrate.

In the present study we have repeated our previous experiments, extending them to include older animals. The ventral prostates of castrates were compared with those of adrenalectomized castrates as well as with those of adrenalectomized and normal controls. Gross prostatic weights were analyzed statistically. The functional state of the ventral prostate was determined by the presence of light areas in the acinar epithelium and these cytological findings were evaluated quantitatively. The data show that the castrate prostates are maintained by the adrenal in the 26 and 31 day groups, but not in the 36 and 41 day groups. Removal of the adrenals alone does not alter the condition of the prostates. This indicates that in the intact immature rat the adrenal does not produce a detectable quantity of androgen, but in the castrated rat up to 31 days the adrenal is capable of producing a physiologically effective quantity of androgen.

The direct measurement of thermal conductance of the skin as an index of peripheral blood flow. ALAN C. BURTON. Johnson Foundation, University of Pennsylvania, Philadelphia.

The temperature of the skin depends upon the ease with which heat flows from the interior of the body to the point of measurement, and thus upon the total thermal conductance of the peripheral tissues. By using the room temperature and the deep body temperature as well as the skin temperature, the total thermal conductance may be calculated. However, an instrument has been developed which measures directly the thermal conductance of the skin, which controls an important part of the total thermal path.

A thin silver disc is separated by an insulating space from a concentric silver ring. Behind each of these lies a flat coil of wire the resistance of which varies with its temperature. The coils form two arms of a Wheatstone's network, so arranged that the current produces more heat behind the disc than behind the ring. The disturbance from balance of the network depends on the excess temperature reached by the coil of the disc over that of the ring. This varies with the thermal conductance of the surface with which the instrument is in contact. Values of the thermal conductance are read from the deflections of a galvanometer after calibration has been made on materials of known conductivity.

Used on the human skin, the instrument shows that while skin temperatures differ widely in different parts, skin thermal conductances are more uniform. Maximum values obtained in vasodilation are greater than those deduced from skin temperature and calorimetric data. Correlation with blood flow is possible in the case of the finger.

The method gives results that are more easily interpretable in terms of the local circulation than does the skin temperature and has many advantages for clinical investigation.

Respiratory modification of the cardiac output. DANIEL H. CAHOON (by invitation), VICTOR JOHNSON and I. E. MICHAEL (by invitation). Department of Physiology, University of Chicago, Chicago, Ill.

The stroke volume of barbitalized dogs breathing normally was recorded

by means of a cardiometer placed over the heart through an opening in the chest wall, which was subsequently sealed airtight. During inspiration the stroke volume was diminished (as has been reported previously). But (contrary to previous reports) the diastolic size of the ventricles also decreased in inspiration, pointing to a diminished ventricular filling in this phase of respiration. The chest of the animal was so prepared that the conditions of pneumothorax and of normal respiration could be produced at once, and alternated at will. Sudden pneumothorax almost completely abolished the changes in stroke volume.

By means of windows in the chest wall, sealed airtight to permit normal respiration, motion pictures were made of the changes in size of the two auricles during respiration. The right auricle increased appreciably in size in inspiration. In expiration it suddenly collapsed, accounting (at least in part) for the increased diastolic ventricular size and ventricular stroke volume at this time. The left auricle was observed to undergo opposite changes in size as regards inspiration and expiration. This effect was not constant however, for on some occasions the left auricle either underwent the same changes as the right, or exhibited no appreciable change in size during respiratory movements.

The middle lobe of the right lung was exposed and the hilus of this lobe ligated, leaving only the bronchus patent. The right auricle was also ligated at the auriculo-atrial junction, isolating it from the venous return to the right heart. Cannuli were then tied into the right auricle and into a pulmonary vein at the periphery of the right middle lobe. Optical recordings made subsequent to reinstitution of normal respiration revealed that in inspiration both the auricle and pulmonary vein increased appreciably in size, indicating an accelerated venous return to the right heart as well as increased blood storage in the pulmonary circuit. During expiration the auricular and pulmonary vein sizes were decreased.

During the exaggerated breathing movements following double vagotomy, the above effects were all accentuated.

Changes in albumin: globulin ratios following intravenous saline injections.

D. BAILEY CALVIN, Department of Biological Chemistry, University of Texas, School of Medicine, Galveston.

Further studies have been made on variations in blood constituents following massive intravenous administration of isotonic and hypertonic sodium chloride and glucose solutions. The observed increases in total circulating plasma proteins, as previously reported, have been amply confirmed in these types of hydremic plethora.

Recent work (Ansel Keyes) on plasma osmotic pressure as a function primarily of albumin concentration made a study of the albumin:globulin ratio desirable. Plasma volumes were determined using dye T 1824 and a modified Gregerson technique. Globulins were precipitated by using twenty-one per cent sodium sulphite (Campbell et al.). Care was taken to standardize conditions of temperature, filtration, etc. Nitrogen determinations were made by the Kjeldahl-distillation technique.

The following results were obtained: *a*, following injection, the A:G ratio rose, from ± 1.6 to as high as 3.3 in one case; *b*, the maximum rise in A:G ratio came within the first fifteen to thirty minutes after injection was completed, but a small response within one to two minutes was usually

observed; *c*, a majority of the rise in *total* circulating protein could be accounted for by increase in total circulating albumin; *d*, comparable results were obtained using isotonic and hypertonic glucose solutions instead of saline solutions for producing hydremia; *e*, chloride of the injected solutions disappeared from the circulation faster than did the water, following use of isotonic solutions.

In vitro experiments were performed to discover whether these increases in A:G ratios could have resulted from *a*, the effects of dilution, or *b*, a possible "dissociation" of plasma globulin to yield albumin-like bodies. The results obtained show conclusively that changes in A:G ratios here reported are real, rather than due to changes in plasma protein nature brought about by dilution, or to errors inherent in the methods used for analysis.

The data suggest a possible entry of plasma protein (primarily albumin) through the capillary wall itself, although this reasoning is open to some objection. The liver is probably a contributing factor. Whatever the source of the extra protein, the net result is an increased effective colloid osmotic pressure, tending to compensate for the marked fall following injection.

The insulin content of the pancreas as influenced by anterior pituitary extract and insulin. JAMES CAMPBELL (by invitation), R. E. HAIST (by invitation), A. W. HAM (by invitation) and C. H. BEST. Departments of Physiology and Anatomy, University of Toronto, Toronto, Canada.

Recent experiments have shown that the daily administration of anterior pituitary extract to normal dogs causes a fall in the insulin content of pancreas. In such animals blood sugar is increased and glycosuria occurs. Experiments have been carried out to determine the influence of insulin on these changes produced by anterior pituitary extract. In one experiment, a group of 4 dogs (group A) was given daily injections of anterior pituitary extract while a second group of 4 dogs (group B) received similar injections of anterior pituitary extract and, in addition, large daily doses of protamine zinc insulin. After 7½ days the insulin contents of the pancreases of group A varied from 0.14 to 0.47 unit per gram. In group B the insulin contents were 2.6, 2.6, 2.2 and 0.23 units/gram. In group B, the dog having the low insulin content showed considerable hyperglycemia and glycosuria in spite of the administration of insulin.

In another experiment, 5 dogs were treated for 11 days. Two of these animals received anterior pituitary extract alone. One of the dogs responded well, showing hyperglycemia and glycosuria. Its pancreas contained 0.38 unit/gram and the islets showed marked hydropic degeneration. The other did not show any diabetic response and the insulin content of its pancreas was 4.1 units/gram. At the same time 3 dogs were given daily doses of anterior pituitary extract and of insulin. Although two of these animals showed some hyperglycemia and glycosuria despite the insulin, yet the insulin contents of their pancreas were 2.3, 1.5 and 2.2 units/gram at the end of 11 days and the islets of Langerhans did not show the marked hydropic degeneration noted in the 11-day animal receiving anterior pituitary extract alone and in others observed previously for the same period.

It would appear that the administration of insulin tends to hinder the fall in insulin content of the pancreas and the degeneration in islet cells

which results from injection of anterior pituitary extract but a final conclusion on this point must await the results of further studies which are now in progress.

Physiologic and pharmacologic studies on the hypothalamus. H. B. CARLSON (by invitation), C. W. DARROW and E. GELLHORN.¹ Department of Physiology, College of Medicine, University of Illinois, Chicago.

The hypothalamus of anesthetized cats (choloralosane 70-100 mgm./kgm.) was stimulated with a Harvard inductorium by bipolar electrodes inserted with the Horsley-Clark apparatus. The cervical sympathetic nerve was cut on one side. The pupillary and nictitating membrane responses were compared on the two sides. In addition to the well known signs of sympathetic excitation, parasympathetic inhibition has been found to result from hypothalamic stimulation as indicated by the dilatation of the sympathetomized pupil. Comparison of the nictitating membrane response to hypothalamic stimulation with the response to stimulation of the cephalad end of the cut cervical sympathetic allowed one to separate central from peripheral effects. Under these conditions, it has been found that on inhalation of gases with low oxygen or increased CO₂-content, the central response greatly increases. Similar effects are obtained with convulsant drugs such as picrotoxin, coramine and particularly metrazol. These effects occur even with subconvulsive doses and in some cases extend over hours.

The effects of different temperatures on the toxicity of rattlesnake venom following its injection into laboratory animals. EMMETT B. CARMICHAEL. School of Medicine, University of Alabama, University. (Read by title.)

The toxicity of rattlesnake venom, when it was injected subcutaneously into rats, was found to vary in summer from that in winter in this laboratory. Following the injection of the venom, the animals were kept at room temperature which usually varied from about 80° to 98°F. during the summer months and from about 48° to 82°F. during the winter months.

It was decided to control the temperature within a limited range which would be more or less comparable to the temperatures experienced during the above seasons. Two temperature ranges were selected: 1, 90° to 98°F. (incubator) and 2, 48° to 60°F. (refrigerator). The rattlesnake venom was dissolved in isotonic saline and injected subcutaneously.

The toxicity of rattlesnake venom for rats is greater when the animals are kept at the higher temperature range. The minimum fatal dose for rats kept at the lower temperature range was about two to three times the minimum fatal dose for those animals that were kept at the higher temperature range. The series of experiments have included 192 rats that were subjected to the lower temperature range and 233 rats that were kept at the higher temperature range.

Guinea pigs, dogs, cats, and monkeys are being used to test the toxicity of rattlesnake venom under the above conditions.

The effects of hexoses on respiratory quotients (R. Q.) of goats. THORNE M. CARPENTER and ERNEST G. RITZMAN (by invitation). Nutrition Lab-

¹ Aided by grant from John and Mary R. Markle Foundation.

oratory, Carnegie Institution of Washington, Boston, Mass., and University of New Hampshire, Durham.

The respiratory quotients of 5 female and 4 male goats (average weight, 38.8 kgm.) were determined after ingestion of 250 cc. of water and before and after ingestion (oral) of 25 grams each of dextrose, levulose, and galactose. Food was removed from the goats 40 hours before each experiment. The amount of sugar given to the goats was calculated from the amount of sugar commonly given in experiments with humans (50 grams) and the relation of the basal metabolism of the goat to that of man. The measurements were made in half-hour periods by the open-circuit chamber method with gas analysis and were continued for 4 hours after ingestion. Water produced a slight rise in the R. Q., galactose caused a maximum rise of 0.05 in the 6th and 7th half hours after ingestion, dextrose a maximum rise of 0.07 in the 6th half hour, and levulose a maximum rise of 0.09 in the 3rd half hour. The curve of the goat's R. Q. of dextrose resembles more nearly that of man than those of the monkey, the canary, and the rat, the other animals thus far studied from this same standpoint. The curve of the goat's R. Q. of galactose resembles most nearly that of the monkey and the curve of levulose most nearly those of man, monkey, and canary. Demonstrable, although slight, amounts of methane were found after the ingestion of the sugars. The amounts, however, would play no significant rôle in either the character or the amount of the total metabolism.

On the mechanism of epinephrine hyperglycemia. R. CARRASCO-FORMIGUERA (introduced by W. B. Cannon). "La Casa de España en México," México, D. F.

In 1933 Bieto and I confirmed by intravenous injections of epinephrine the results from subcutaneous injections (Cori) showing no noteworthy difference of the glycemia concentration between arterial and venous blood. Whereas an intrasaphenous injection, however, elicited hyperglycemia without marked arterio-venous difference, the same dose injected into the portal system was followed by a hyperglycemia having an arterio-venous difference similar to that seen after glucose ingestion.

Recently Griffith and co-workers have reported a range of optimal rate of epinephrine injection within which the hyperglycemia is proportional to the dose, and above which it decreases as the dose increases. One of their suggestions, that there is a local action on peripheral tissues, accords with the interpretation which Bieto and I offered for our results.

Soula's recent emphasis on the skin as a reservoir for glucose raised the question whether epinephrine might have local effects through its local vascular action. Accordingly experiments were planned (a) to compare the hyperglycemias resulting from different doses of epinephrine introduced through the saphenous or portal vein, and (b) to compare glucose concentrations in the skin under these conditions. Work is in progress in these lines.

Six normal dogs, fasting 24 hours, were injected with adrenalin, as follows. Two were given a single injection, 0.025 mgm. per kilo. Two were injected via a saphenous vein, at half-hour intervals, three times; twice with 0.014 mgm., the third time with 0.028 mgm. per kilo. Two received 0.025 mgm. per kilo via a saphenous vein, and an hour later the

same dose via the portal. Blood sugar was determined in arterial and venous samples taken 30 and 5 minutes before the first injection, 3 and 30 minutes after each injection, and also 60 minutes after the first injection in the third pair (above).

The results confirmed the observations reported by Bieto and myself, mentioned above. Three successive injections of adrenalin within an hour were each followed by hyperglycemia, with or without an arterio-venous difference, according to whether the injection entered via the portal or via a peripheral vein. The degree of hyperglycemia elicited by successive doses of adrenalin requires further investigation.

Excretion of gonadotrophic hormone by prepuberal and adolescent girls.

H. R. CATCHPOLE and W. W. GREULICH (introduced by J. F. Fulton).

Laboratory of Physiology and Department of Anatomy, Yale University School of Medicine, New Haven, Conn.¹

Two groups of subjects were available for this study: a group of 8 symptom-free patients from a sanatorium for tuberculosis, on 4 of whom repeat studies were made after a lapse of one year, and a group of 4 normal girls. Assays were made of the daily excretion of follicle stimulating hormone over a nominal period of about a month by a technique already reported (Catchpole, Greulich and Sollenberger, *Am. J. Physiol.*, **123**: 32, 1938). A wide fluctuation in hormone excretion was found from day to day in the same subject, as well as widely differing total pictures for the several subjects. The latter could be arranged in a sequence from those showing little or no hormone excretion over the experimental period to those in which hormone was rather generally found. An attempt was made to correlate developmental status with hormonal findings. Definite immaturity was associated with absence (less than 2 M.U. per day) of hormone over consecutive periods of several days, with occasional "escape" values on individual days of 3 to 7 M.U. per day. The majority of the subjects fell into an intermediate group in which hormone was detected more frequently over the collection period and in which the daily excretion then ranged from 3 to 10 M.U. The frequency of appearance and the amounts of hormone recovered (up to 20 M.U.) intensified in subjects approaching menarche, as was well shown in two of the four subjects on whom repeat studies were made a year later. Pituitary gonadotrophic activity, as shown by the excretion of an active hormone, is demonstrable for a considerable period (at least a year) prior to menarche, and the latter is associated with progressive increases in amounts of hormone, and with the frequency with which it can be detected in the urine.

The influence of cutaneous atmospheric oxygen absorption upon the apparent total oxygen utilization of the body. ALFRED H. CHAMBERS (by invitation) and SAMUEL GOLDSCHMIDT. Laboratory of Physiology, University of Pennsylvania Medical School, Philadelphia.

Respiratory oxygen absorption was determined by the Tissot method on subjects enclosed up to the neck in a metal box. The gaseous atmosphere surrounding the body could be changed at will.

Two of four subjects increased their oxygen consumption from the in-

¹ This work was aided by a grant from the Rockefeller Foundation to the Adolescence Study Unit, Yale University School of Medicine.

spired atmospheric air up to 10 per cent, or more, when nitrogen replaced the room air surrounding the body. In the other two subjects the rise, under similar experimental conditions, was either entirely absent, upon occasion, or, at other times, increased to a slight degree.

The results can be explained on the assumption that the oxygen for cutaneous metabolism has a double source. It may come either from the atmospheric or from the blood oxygen. When deprived of the atmospheric source an increased utilization from the blood is evidenced.

Serological and physical properties of films of streptococcal proteins. LESLIE A. CHAMBERS, J. B. BATEMAN and H. E. CALKINS (introduced by D. W. Bronk). Johnson Foundation, University of Pennsylvania, Philadelphia.

The two sides of films of a nucleoprotein antigen prepared from hemolytic streptococci have been found (Chambers) to differ in the degree to which they react with various homologous and heterologous antisera.

The conditions for complete spreading of the nucleoprotein (NPA) and of a phosphorus free derivative (P_6) were determined by the method of Bateman and Chambers. Completely and incompletely spread films of each substance were then deposited from various buffered substrates.

Completely spread films (monomolecular?) of P_6 showed a sharp differentiation between the two faces, while those of NPA showed the same orientation of reactive sites but to a lesser degree.

The reaction with homologous anti-NPA serum was always strongest on the air (AB) surface of the film; that with homologous antistreptococcal serum was equally strong on both faces. On the other hand, heterologous anti-NPA serum failed to combine with either surface, while heterologous antistreptococcal serum sensitized the water face (A) predominantly.

The facial differences were well marked in films spread at pH 4.5 and pH 7.0; they were less well defined in the pH 8.0 case. In films spread on 0.01M HCl (pH 2.0) the reaction with both homologous anti-NPA and heterologous antistreptococcal sera occurred on the AB face of the film, as did that with homologous antistreptococcal serum. It is concluded that the two reactive sites behave independently insofar as their localization is governed by pH of the substrate.

Incompletely spread films showed no measurable facial differences; failure to permit orientation of the molecules also prevented localization of the reacting sites. Film thickness measurements showed the degree of differentiation between the two faces to be related to the extent of spreading permitted.

It is concluded from the similar behaviors of NPA and P_6 films that the component of NPA which entered the film structure was the protein, and further, that nucleic acid plays no role in the film formation or in the serological reactivity of the deposited films.

Riboflavin and the photochemical oxidation of Cypridina luciferin. AURIN M. CHASE. Physiological Laboratory, Princeton University, Princeton, N. J.

It has been observed by Harvey (1925) that the light emission of a solution of *Cypridina* luciferin and luciferase is suppressed by brief irradiation with the carbon arc. He showed that the effect of the irradiation was upon the luciferin and attributed it to a rapid photochemical non-lumines-

cent oxidation of luciferin. Various fluorescent dyes enhanced the effect, probably through photosensitization, since previously ineffective wavelengths could be made effective by adding appropriate dyes.

In a recent repetition of some of this work it was found that a colored component of luciferase extracts was apparently responsible for this photochemical effect upon luciferin. By using relatively colorless luciferase extracts and partially purified luciferin, luminescence occurred which was not quenched by irradiation with the carbon arc. Addition of highly colored, boiled luciferase extracts, followed by irradiation, resulted in suppression of luminescence.

Addition of Merck's riboflavin to a mixture of partially purified luciferin and relatively colorless luciferase extract (whose luminescence was not previously quenched by irradiation) resulted in marked suppression of luminescence upon irradiation. The quantity of riboflavin which produced a marked effect was about 0.00003 per cent in the luciferin-luciferase solution of pH 6.6.

Dialysis of a one per cent crude aqueous luciferase extract against distilled water changes the pH from 8.6 to 5.0 with precipitation of material containing about ten per cent of the luciferase activity. The almost colorless supernatant has about fifty per cent of the original luciferase activity and its absorption spectrum shows a broad band at 270-280 m μ . The dialysates show strong absorption at about 270 m μ (probably due to amino acids) and very slight absorption maxima at about 360 and 450 m μ , believed to indicate free flavin which may act as a sensitizer for the photochemical oxidation of luciferin upon irradiation. Luciferin solutions change in light absorption during exposure to air, indicating a possible basis for a photochemical oxidative effect.

Chemical properties of intermedin. BACON F. CHOW (by invitation), R. O. GREEP (by invitation), and H. B. VAN DYKE. Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick, N. J.

When hog whole pituitary glands, freshly ground, are extracted with 2 per cent NaCl, a part of the intermedin is dissolved in the salt solution together with other pituitary hormones. It was found that melanosome-dispersing hormone (intermedin) easily passes through a cellophane membrane. Thus, it can be separated by dialysis from all proteins. It can then be concentrated by evaporating to dryness over a steam bath without apparent destruction of its activity. The hormone can be separated from inorganic salts by dissolving it in an acid-alcohol solution.

The intermedin remaining in the 2 per cent NaCl extracted residue can also be liberated by boiling in 0.1 N H₂SO₄. The protein in the acid extract may be removed either by precipitating with trichloroacetic acid or by shaking with chloroform. The protein-free solution is highly active.

The melanosome-dispersing activity of the hormone was tested in either light-adapted or hypophysectomized frogs. It was found to be destroyed by digestion with crystalline proteolytic enzymes (trypsin, chymo-trypsin, pepsin, etc.) and by prolonged boiling in either acid or alkali, indicating that it is a polypeptide. The distribution of amino acids of the hormone extract was determined.

It was found that the active substance can pass through parchment paper. It is therefore suggested that intermedin is of comparatively low molecular weight.

The experimental use of lipocaic in the treatment of psoriasis. DWIGHT E. CLARK (by invitation), EDMUND WALSH (by invitation), ORMAND C. JULIAN (by invitation) and LESTER R. DRAGSTEDT. Departments of Surgery and Dermatology, The University of Chicago, Chicago, Ill.

The rôle of lipid metabolism in relation to psoriasis has been the subject of numerous studies. Many investigators have shown that a hyperlipemia exists in about 70 per cent of patients with clinical evidence of psoriasis and reported beneficial effects with low fat diets.

In 1936 Dragstedt, Prohaska and Harms showed that the pancreas manufactures in addition to insulin another hormone which seems to be concerned in some way with the metabolism of fat. They found that 1 to 1.5 gram of a neutral alcohol extract of pancreas, rendered free of fat by extraction with ether, was effective in preventing the deposition of fat in the liver of depancreatized dogs. Sufficient evidence, however, has not yet been obtained as to whether lipocaic, which they believed to be the active principle of the pancreatic extract, has to do with oxidation of fat or is mainly concerned with fat transportation.

This investigation was suggested by the finding that lipocaic produces a definite decrease in the blood lipids of patients with xanthoma.

Twelve cases of psoriasis are presented which have been treated with lipocaic and observed from 5-14 months. About 50 per cent of the patients presented an initial hyperlipemia. Lipocaic tended to lower the blood lipids in all the cases but no definite correlation between the blood lipid level and the clinical condition could be made. The patients with the highest blood lipids seemed to be the most resistant to treatment.

Three patients failed to show some improvement following the daily oral administration of lipocaic. About 50 per cent of the patients showed almost complete disappearance of the lesions with the lipocaic alone. The remaining cases which improved somewhat with lipocaic alone cleared more rapidly than usual when local treatment was supplemented.

Lipocaic seems to be a valuable adjunct in the treatment of psoriasis.

Electrical axis and monocardigrams in normal students. PAUL CLARK (by invitation), ROBERT C. ROBB (by invitation) and JANE SANDS ROBB. Student Health Service and Department of Pharmacology, Syracuse University, Syracuse, N. Y.

Electrical axis calculated from three simultaneously recorded leads has been discussed previously in the American Heart Journal 14: 588, 1937. The apparent axis has been calculated throughout the cycle and graphs constructed. Monocardigrams have been derived by the formula suggested by Mann (Arch. Int. Med. 25: 283, 1920). While all monocardigrams lie in the general position described by Mann for normals, there are considerable variations from graph to graph.

The effects of salts and adrenal cortical extract upon toxicity of drugs. WILLIAM G. CLARK and RICHARD H. BARNES (introduced by Maurice B. Visscher). Departments of Zoology and Physiology, University of Minnesota, Minneapolis.

Laszt (Nature 144: 244, 1939) has recently shown that the effects of iodoacetate poisoning on the rate of intestinal absorption of glucose and on the general toxicity of iodoacetate in the rat, are ameliorated by treatment with NaCl. This finding seemed to support the analogy drawn by the Verzáz group between iodoacetate poisoning and adrenal insufficiency. The present study was undertaken to investigate the effects of NaCl, KCl, and adrenal cortical hormone upon the toxicity of iodoacetate poisoning and upon the toxicity of drugs known to exert toxic symptoms in no way associated with the specific enzyme processes postulated to be impaired in adrenal insufficiency.

Iodoacetate administered subcutaneously to rats, produced severe gastrointestinal hemorrhages, diarrhea, hemorrhagic nephritis, a marked lowering of body temperature, and death. Oral NaCl (with citrate or bicarbonate in small amounts) was definitely beneficial in maintaining body temperature and life. Oral KCl aggravated the toxicity of iodoacetate, but large doses of adrenal cortical extract had no effect. The toxic alkaloid, colchicine, produced the same symptoms as described above for iodoacetate. Orally administered NaCl and KCl both exerted a favorable effect against the fall in body temperature, and prolonged the survival of colchicine poisoned rats. Massive doses of cortical extract, however, exerted an even greater protection against colchicine toxicity.

The results will be discussed in the light of the use of iodoacetate as a poison inhibiting specific enzyme processes, supposedly suppressed by the lack of the adrenal cortical hormone.

Distribution of auditory action-potentials in the medial geniculate body of the cat. JOHN D. COAKLEY (by invitation) and ELMER CULLER. University of Rochester, Rochester, N. Y.

In a former report we described a technique for recording neural impulses of the acoustic system at the level of the thalamus and showed by numerous tests that the potentials recorded and measured were generated by the cells and fibers of the medial geniculate body (m.g.b.). The purpose of the present study was to determine by electric recording whether the various audible frequencies involve separate pathways in this nucleus and, if so, where these are located.

Five test-frequencies (125, 250, 500, 1000 and 2000 cycles) were applied successively to the animal's ear and the neural potentials measured. With the aid of a stereotaxic instrument measurements were made at 112 points distributed uniformly throughout the nucleus and points just beyond its margins. However, only a portion of these points could be studied in any one of the 21 cats used. A measurement for each stimulus-frequency was obtained at each of the 112 points from 12 different m.g.b.'s. and the average of these 12 measurements was taken as a measure of the potential developed at any point.

Computation of the mean positions of the potentials revealed that the fibers entering m.g.b. are functionally differentiated: fibers near the center of the bundle are active when the stimulus frequency is low and the other fibers are spirally arranged about this center as a function of frequency of stimulation. This spiral arrangement is particularly apparent in horizontal sections of the nucleus.

Thus it is concluded that: 1. Neural impulses initiated by different stimulus-frequencies traverse the medial geniculate body of the cat by

different routes. 2. The pathways followed by the neural impulses are arranged spirally as a function of the stimulating frequency. This formation appears as the fibers emerge from the brachium of the inferior colliculus and continues in slightly modified form through the nucleus. 3. Two correlates of the frequency of stimulation appear at this level of the acoustic system: specificity of fibers involved and specificity of the frequency pattern of the neural potentials. It is suggested that both of these correlates of the stimulus-frequency may participate in determination of pitch.

*A comparison of the histamine content of blood and bone marrow.*¹ CHARLES F. CODE and JAMES L. JENSEN (by invitation). Department of Physiology, Medical School, University of Minnesota, Minneapolis.

In an earlier study (J. Physiol. **90**: 349, 1937) the white blood cells were found to be the major source of the histamine contained in normal blood. A later investigation (J. Physiol. **90**: 485, 1937) allowed the conclusion that of the white cells present in blood the granular or myeloid series contained most of the histamine. The question arose as to whether the myeloid cells contained histamine when they left the bone marrow or obtained it later in the peripheral circulation. To gain some information on this point we have determined the histamine content of the bone marrow and compared it with that of the blood in several animal species. In the dog, cat, rabbit and guinea pig the bone marrow histamine content has been consistently in excess of that found in the blood. It seems possible that the leucocytes contain some histamine when they leave the bone marrow.

The effect of estrin on basal metabolism. MARY E. COLLETT, FAITH W. REED (by invitation), SYLVIA ROUSE (by invitation), ELEANOR YEAKEL (by invitation) and LEABELLE ISAAC (by invitation). Department of Biology, Western Reserve University, Cleveland, O. (Read by title.)

Six women who had undergone surgical removal of uterus and both ovaries were given small to moderate doses of estrin orally or intramuscularly daily for 6 days. From 2 to 5 series of doses were given each patient. The basal metabolism rose above the initial level during the week of medication on the smallest doses (500 IU estrone injected), but on larger doses (1000 to 2000 IU injected) rose somewhat less or fell for a time, and rose again in the week following medication. Equivalent effects upon BMR were obtained with 1000 IU injected estrone, 2000 IU oral estrone, and 4000 to 6000 IU oral estriol. Estrone, by this test, is more potent than estriol if both are given orally. The effectiveness of oral administration as compared with injection is greater than might have been anticipated. We are indebted to the Department of Obstetrics and Gynecology for subjects, and to the Council on Pharmacy and Chemistry for a grant covering part of the cost of the research.

Pressor effects from restoration of renal circulation after periods of complete ischemia. DEAN A. COLLINS and ANGIE S. HAMILTON (by invitation). Department of Physiology, Temple University School of Medicine, Philadelphia.

¹This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

If the circulation through the kidneys is arrested for relatively long periods (24 hrs.—Dicker; $5\frac{1}{2}$ to $6\frac{1}{2}$ hrs.—Taquini), a sustained elevation of blood pressure occurs upon restoration of the circulation. We have studied this phenomenon in 40 dogs. The animals were anesthetized with chloralose, and blood pressure was recorded from the femoral artery with a mercury manometer. After separation of the kidneys from surrounding tissue and destruction of blood vessels along the ureter, the renal arteries and veins were occluded by clamps, which could be operated after closure of the abdomen. Manipulation of these clamps in control experiments gave no change in blood pressure. The findings of Taquini were confirmed: release of the clamps after $5\frac{1}{2}$ to $6\frac{1}{2}$ hours of complete ischemia resulted in a sustained rise of blood pressure (average = 35 mm. Hg).

We have found that shorter periods of complete renal ischemia gave similar, although usually somewhat less marked, effects. Complete ischemia for a period as short as $\frac{1}{2}$ hour resulted in an elevation of blood pressure. After unclamping, there was often a temporary rise and fall preceding a sustained rise lasting for the duration of these acute experiments (over an hour in many cases).

Occlusion and subsequent restoration of the blood supply of one kidney (with the other intact or with its vessels clamped) was followed by an elevation of blood pressure.

Good responses were obtained after the following procedures: 1, denervation of the kidneys; 2, destruction of the cord below the lower cervical segments combined with vagotomy; 3, splenectomy; 4, adrenalectomy.

This phenomenon appears to be specific for the kidney. Complete ischemia of liver or leg was followed by either a fall or no change in blood pressure. Elevations of blood pressure were obtained in the case of the spleen, but these were found to be due to volume changes of this organ.

Effects of thiocyanates on blood pressure. D. A. COLLINS, J. LANSBURY (by invitation) and M. J. OPPENHEIMER (by invitation). Laboratory of Physiology and Department of Medicine, Temple University, School of Medicine, Philadelphia, Pa. (Read by title.)

In the use of thiocyanates some clinicians find that patients with high blood pressure fall into two groups, one responding with a marked fall in blood pressure, the other poorly or not at all. It seemed of interest to determine how experimental hypertension, produced by constriction of the renal arteries, responds to thiocyanate. The effects of thiocyanate on normal dogs were studied as a first step.

Nine courses of potassium thiocyanate (per orem), varying in length from 5 to 38 days, were given to 6 normal dogs. The blood pressure was determined on the hind leg by the auscultatory method of Allen. Plasma or serum thiocyanate was determined by Schreiber's technique as modified by Barker.

With the plasma levels ordinarily employed clinically (8-12 mg. per 100 cc.) no significant falls were obtained. With values ranging from 15 to 35 mgm. per 100 cc. falls were obtained in some cases, but were always accompanied by various toxic symptoms.

Doses varied from 20 to 70 mgm. per kgm. per day, except in one case where death resulted after doses up to 135 mgm. per kgm. per day.

The reaction of a series of hypertensive dogs to thiocyanates is being studied at the present time.

Medullotropic and antidiabetogenic effects of pituitary extracts prepared from a primary alcoholic extract of fresh gland tissue. J. B. COLLIP. Department of Biochemistry, McGill University, Montreal, Quebec, Canada.

It has been shown that the primary alcoholic extract of fresh pituitary tissue contains a substance or substances which are of hormone nature and which are active when administered orally to experimental animals or to man.

The results of a large series of experiments on animals and some patients in which changes in blood sugar level have been produced will be described and their significance discussed in relation to carbohydrate metabolism and diabetes.

Vagal tonicity as related to body temperature in the snake. RUTH E. CONKLIN (introduced by Cecil K. Drinker). Vassar College, Poughkeepsie, N. Y. (Read by title.)

Anesthetized snakes were subjected to changes of body temperature by the use of water circulating through gooch tubing on either side of the snake. Vagotomy did not cause any significant change in heart rate either at room temperature or at 8° above or 5° below room temperature. Obviously, the tonus of the cardiac vagus is low. When, however, body temperature was slowly raised to approximately 37°C. and then lowered, with repetition of the procedure after vagotomy, the rate reached at the highest temperature after vagotomy showed an average increase of 14%. Atropine had the same effect. A control experiment with two heating periods before vagotomy showed no increase in rate during the second period over the first, but the usual increase after vagotomy. Since in the frog the sympathetic nerve endings in the perfused heart have been found much more resistant to heat than the vagus endings (Freyer and Gellhorn, *Am. J. Physiol.* **103**: 392, 1933), it seems possible that the same thing is true of the snake, and that slight tonic activity of the vagus only masks the greater activity of the sympathetic, especially when the latter is increased by heat.

The effects of angiotonin on renal blood flow and on the excretion of phenol red and inulin in the dog. A. C. CORCORAN (by invitation) and IRVINE H. PAGE. Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind.

Renal blood flow, calculated from the simultaneous renal clearances and extraction ratios of phenol red and inulin was determined in uninephrectomized female dogs with subcutaneously explanted kidneys during the slow infusion of a solution of angiotonin, the pressor substance formed by the interaction of renin and renin-activator.

Infusion of angiotonin was found to result in increased arterial pressure, decreased renal blood flow and increased renal extraction of inulin. Renal excretion of phenol red was not specifically affected.

It is concluded that the action of angiotonin on the kidney is similar to that of renin and that it is in great part due to constriction of glomerular efferent arterioles. The action of angiotonin, however, is more transient than that of renin and ceases immediately when the infusion is withdrawn.

Renal excretion of diodrast in the dog. A. C. CORCORAN (by invitation), IRVINE H. PAGE and H. W. SMITH. Lilly Laboratory for Clinical Research, Indianapolis City Hospital, and Department of Physiology, New York University. (Read by title.)

The proportion of diodrast removed from renal blood by the subcutaneously explanted kidneys of uninephrectomized female dogs was determined at concentrations of 0.4 to 15.0 mgm. diodrast (expressed as iodine) per 100 cc. of plasma. Renal plasma flows were found by calculation from the simultaneous renal clearances and extraction ratios of diodrast, phenol red and inulin.

The separately determined renal plasma flows agreed within the errors of the methods.

The load of diodrast presented the kidney was calculated as mg. per square meter of body surface per minute. The proportion of diodrast removed from blood by the kidney decreased slowly from a maximum of about 90 per cent as the amount of diodrast-iodine presented the kidney exceeded 1.0 mgm. per square meter per minute.

It is concluded that the mechanism of excretion of diodrast in the dog differs somewhat from that which obtains in man.

Renal hyperemia due to uninephrectomy and the possible presence of blood in the renal vein which had not come into contact with renal tubular tissue may explain the failure to observe complete removal of diodrast from blood when less than 1.0 mgm. diodrast-iodine per square meter apparently entered the kidney each minute.

Comparative effects of progesterone and cortico-adrenal extract on carbohydrate metabolism in the rat. E. L. COREY. Physiological Laboratory, University of Virginia Medical School, University. (Read by title.)

Following observations indicating that progesterone¹ may maintain the lives of adrenalectomized male cats when substituted for cortico-adrenal extract, determinations of comparative effects on blood-sugar, liver and muscle glycogen levels were made in normal, fasted, hypophysectomized and adrenalectomized rats. In most (45) instances 5 mgm. of progesterone in oil or 5 cc. of the extract were injected subcutaneously, and blood and tissue samples taken 6 hours later.

In normal fasted and unfasted animals (28), no significant differences could be seen between carbohydrate levels of progesterone and saline-injected controls. Cortico-adrenal extract alone produced significant elevations in blood-sugar and liver glycogen. Rats (18) adrenalectomized for 4 days exhibited no significant changes following progesterone injection, although cortico-adrenal extract restored normal carbohydrate values. Carbohydrate levels in rats (8) hypophysectomized for 7 days and injected hourly with 1 mgm. of progesterone, 1 cc. of saline solution or 1 cc. of the extract showed carbohydrate restoration only in those treated with our cortical preparation.

Under the conditions imposed, no evidence could be found of any progesterone-induced influences on carbohydrate metabolism in the rat, in contradistinction to observations following cortico-adrenal extract administration.

¹ "Proluton," synthetic, crystalline progesterone furnished to us through the generosity of the Schering Corporation.

Survival of adrenalectomized cats injected with progesterone. E. L. COREY. Physiological Laboratory, University of Virginia Medical School, University. (Read by title.)

Experiments on the survival of adrenalectomized cats injected with progesterone¹ in oil indicated that otherwise normal and castrated male animals might be resuscitated from symptoms of adrenal insufficiency and be maintained in excellent condition for at least 3 weeks on a dosage of 10 mg. of progesterone daily. Such animals when sacrificed showed blood-sugar, serum sodium, chloride, potassium and urea, as well as liver, muscle and cardiac glycogen levels well within normal limits. Other male animals in which treatment was stopped after 3 weeks showed a recurrence of symptoms of acute adrenal insufficiency and when sacrificed exhibited blood and tissue chemical findings similar to those seen in severe adrenal insufficiency.

On the other hand, female cats adrenalectomized in the spring died under similar progesterone treatment with symptoms and blood-chemical findings similar to those seen in acute cortico-adrenal insufficiency, despite large injections of progesterone. However, castrated female cats survived adrenalectomy for over 3 weeks in excellent health during daily (10 mgm.) progesterone injection.

It is concluded that progesterone, when administered in sufficient amount, may materially prolong the life of adrenalectomized male and castrated female cats. The presence of some other ovarian hormone in adrenalectomized but otherwise normal female cats prevents the effective action of progesterone in such animals.

Effect of cortico-adrenal extract on the perfused liver. E. L. COREY and S. W. BRITTON. Physiological Laboratory, University of Virginia Medical School, University. (Read by title.)

In an attempt to ascertain possible effects of cortico-adrenal extract on the carbohydrate metabolism of the isolated liver, rat and cat livers were excised following cannulation of the hepatic portal vein and perfused in a constant-temperature bath containing isotonic Ringer's solution. Perfusing fluids consisted of 1, Ringer's solution; 2, 5 per cent glucose in Ringer's solution; 3, 5 per cent cortico-adrenal extract in solution 2; and solutions 2 and 3 to which had been added gum acacia in 7 per cent concentration.

In all rat livers the glycogen content of perfused livers was found to fall, and glycogenesis could not be demonstrated. However, the results appeared suggestive of definite action by the extract. Thus, when perfused with solution 1 a loss of 75 per cent of the original liver glycogen content was observed. The addition of glucose in solution 2 reduced the glycogen fall to 35 per cent, and with the addition of cortico-adrenal extract only 21 per cent of the pre-experimental hepatic glycogen was lost.

In perfused cat livers definite evidence of glycogenesis was obtained following perfusion with solution 3 to which gum acacia was added. Thus, in a limited series (5 animals), livers perfused with solution 2 plus the gum showed a fall in pre-experimental glycogen content of 12 per cent during 10 minutes, while in 4 cases perfusion with solution 3 plus gum acacia

¹ "Proluton," synthetic, crystalline progesterone furnished to us through the generosity of the Schering Corporation.

resulted in an average rise of over 80 per cent in liver glycogen during a similar period. This experimental work is being continued.

Studies on the acetylcholine content and on the activity of the cholinesterase in the brain. R. CORTELL (by invitation), J. FELDMAN (by invitation) and E. GELLHORN.¹ Department of Physiology, College of Medicine, University of Illinois, Chicago.

The acetylcholine was extracted with trichloroacetic acid, the filtrate was shaken with ether and the condensed extract was assayed on the eserized rectus abdominis muscle of the frog (Chang and Gaddum, J. Physiol. 79: 255, 1933).

The esterase was extracted with 0.9 per cent sodium chloride and the activity was determined by the continuous electrometric titration method of D. Glick (J. Gen. Physiol. 21: 289, 1937).

The experiments show that the acetylcholine content of the hemispheres is regularly lower than that of the brain stem of the unanesthetized rabbit. Similarly it is found that the cholinesterase activity is greater in the brain stem than in the hemispheres. Prolonged anoxia and insulin hypoglycemia do not alter acetylcholine content or cholinesterase activity. It is, however, found that eserine (1.5 mgm./kgm. intravenously) significantly increases the acetylcholine content of hemispheres and brain stem and reduces the activity of the cholinesterase.

Bile salts and fat absorption. LATHAN A. CRANDALL, JR. and H. BERRY IVY (by invitation). Department of Physiology, University of Tennessee, Memphis.

The rise in plasma fat following the administration of olive oil was used as an index of the rate of fat absorption. When 100 cc. of olive oil plus 150 cc. of water were given by stomach tube to dogs in which the gall bladder had been anastomosed to the renal pelvis and the common bile duct ligated (complete loss of bile into urinary tract), no significant rise in blood fat occurred during the next five hours. If 3 grams of commercial bile salts were dissolved in the water with which the olive oil was given, the rise in blood fat level approximated that of the normal dog. Sodium dehydrocholate appeared to be less effective than commercial bile salts in promoting fat absorption as judged by the increase in blood fat level; data on other bile salt derivatives will also be presented.

Cortical potentials mediated by the corpus callosum. HOWARD J. CURTIS (introduced by Philip Bard). Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

In cats and rabbits single electrical shocks were applied to the cortex of one hemisphere which evoked potentials at one or more points on the cortex of the other hemisphere. These responses are mediated by the corpus callosum. The potentials may raise, lower, or leave unaffected the threshold for electrical stimulation at this point, as indicated by muscle twitches in response to test shocks. This cortical facilitation or inhibition may last for as long as 100 milliseconds after the arrival of the first part of the wave. It has not been possible as yet to predict from the shape of the wave whether inhibition or facilitation will result.

¹ Aided by grant from John and Mary R. Markle Foundation.

The potential wave is typically composed of a surface positive component lasting about 15 milliseconds immediately followed by a surface negative component lasting about 75 milliseconds. If a convulsant drug such as picrotoxin is applied to the surface of the pia, the negative component is greatly increased in magnitude, and the positive component is increased slightly. If an anesthetic drug such as nembutal is applied the negative component is completely obliterated, leaving the positive component unchanged.

Bipolar micro-electrodes consisting of two glass capillaries fused together and filled with saline were inserted into the cortex by means of a modified Horsley-Clark instrument. They were usually about 50 microns in diameter and 200 microns apart, and the potential as recorded at the surface was unaffected by their insertion or movement. Using these electrodes in conjunction with convulsant or anesthetic drugs it has been possible to separate the two components of the potential wave and sharply localize them within the cortex. These data seem to indicate that the ascending fibers of the callosum ramify profusely in the deeper layers of the cortex and send fibers to the surface. Here they make synaptic connection with descending internuncial neurons which go to the deeper cortical layers. The ascending fibers give rise to the surface positive component of the wave, and the descending internuncial fibers to the negative component.

Membrane action potentials of the squid giant axon. HOWARD J. CURTIS (by invitation) and KENNETH S. COLE. Department of Physiology, Columbia University, New York City. (Read by title.)

A technique has been developed for recording action potentials of the squid giant axon between one electrode inside and another electrode outside the axon. The membrane at one end of the axon is pierced by a capillary needle electrode, insulated except at its tip, which is then pushed along the axis of the fiber for a distance of about 9 mm. The needle produces only a local injury at the point of puncture which spreads slowly while the rest of the axon remains excitable for as long as four hours in some cases. The action potential recorded between the tip of the needle and an electrode directly opposite it on the outside of the axon is approximately the action potential difference across the membrane. This membrane potential had a usual spike height of about 50 millivolts although 80 millivolts has been found. The membrane spike potential was about five to ten times that obtained from conventional outside "monophasic" leads. When the preparation was fresh, the negative spike of the membrane potential was followed by a slow positive phase with about one-fifth the spike amplitude. This positive phase decreased as injury approached the recording region. The amplitude and form of the membrane action potential was shown to be the same for propagation in either direction.

The estrin-gonadotropin relationship during the menstrual cycle. FRED E. D'AMOUR. The Biologic Research Laboratory, University of Denver, Denver, Colo.

Daily assays of 24-hour urine specimens for both estrin and gonadotropin have been carried out on approximately 25 menstrual cycles in 5 subjects.

Following removal of the tannic acid precipitate (method of Levin and Tyndale) the urine was hydrolyzed and extracted with chloroform. For gonadotropin, 4 or 8 animals were used on each days sample, for estrin 12 or 24.

The most significant finding was that the first estrin excretion preceded the appearance of gonadotropin. It may therefore be that the secretion of hypophyseal gonadotropin which is thought to be responsible for ovulation, itself results from the stimulation of this organ by estrin secreted by the developing follicle.

Administration of estrin to one subject for a period of 3 cycles resulted in a much greater output of gonadotropin during the first cycle, and little effect on gonadotropin during the following 2 cycles, its discontinuance being again followed by an increased output.

A comparison of international gonadotropin standards. MARIE C. D'AMOUR and FRED E. D'AMOUR. The Biologic Research Laboratory, University of Denver, Denver, Colo.

The Health Organization of the League of Nations has now established standards and defined units for the gonadotropin from pregnancy urine and that from pregnant mare serum. These preparations will be widely used in assaying commercial products and some knowledge of the biologic potency of the units seems desirable.

This paper presents the results of assays which lead to the following conclusions:

1. The biologic potency of 1 I.U. of P.U. is nearly the same, by whatever test employed, as that of 1 I.U. of P.M.S.
2. But larger doses, say 20 I.U. of P.M.S. produce greater responses than 20 I.U. of P.U.
3. In assaying preparations against these standards, a combination of the vaginal smear and uterine weight methods seems, for a number of reasons, to be preferable to other methods.

Apparatus for continuous recording of systolic blood pressure. CHESTER W. DARROW. Institute for Juvenile Research and University of Illinois, Department of Physiology, College of Medicine, Chicago. (Demonstration.)

Continuous recording of systolic blood pressure by methods described at the 1937 meeting will be demonstrated by a compact instrument recently constructed by Stoelting & Company. This will be accompanied by actual recording and by sample records.

The relation of electrical conductance of the brain to cerebral function. CHESTER W. DARROW and ERNST GELLHORN.¹ Department of Physiology, College of Medicine, University of Illinois, and the Institute for Juvenile Research, Chicago.

The conductance of the brain at different frequencies has been studied extensively under various conditions by Spiegel and Adolf. We have employed such conductance measurements in experiments on cats (100 mgm. chloralsane per kilo) as an index of functional changes in the brain which are correlated with indications of autonomic and somatic activity.

¹ Aided by John and Mary R. Markle Foundation.

We have found that even *in vitro* a very slight increase in pressure on the brain greatly increases brain resistance (opposite effect after coagulation by heat or formalin) and that in the intact animal even changes in blood pressure tend to produce parallel changes in the resistance of the brain when the electrodes are held rigidly in place. We have, therefore, supported our bipolar electrode by a light spring so that pressure and pressure changes on the tissue are minimal.

With the effects of pressure eliminated, oxygen lack, asphyxia, acetylcholine and amylnitrite are shown to produce increase of brain resistance, whereas adrenalin and metrazol occasion decrease of resistance. When recording action potentials with the electrode placed in the auditory area, it is shown that conditions which increase resistance (decrease conductance) are associated with a decrease of spontaneous potentials and a decrease of the potential response to an auditory stimulus, whereas those conditions which increase conductance increase these potentials. We have, nevertheless, not demonstrated clear differences in the effects of these conditions on conductance measured at different frequencies (100, 1000 and 10,000 cycles).

The secretion of bromide ions by the gastric mucosa. HORACE W. DAVENPORT (introduced by G. H. Whipple). Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Sodium bromide was fed to or injected into dogs, cats and rats. Up to 50 per cent of the chloride of the serum was replaced by bromide. When the gastric mucosa was stimulated to secrete acid the ratio of bromide to chloride in the gastric secretion was always exactly the same as the ratio of bromide to chloride in the serum. Hydrobromic and hydrochloric acids were secreted, and the proportion of each secreted depended only on the proportion of bromide ions and chloride ions in the serum. The gastric mucosa does thermodynamic work in concentrating and secreting the chloride ions, and it is capable of doing exactly the same work in concentrating and secreting bromide ions. It is concluded that the mechanism performing the work is not specific for chloride ions. The most likely explanation is that the mechanism secreting the acid concentrates and secretes hydrogen ions and that the chloride ions accompany the hydrogen ions in order to maintain electrical neutrality. The chemical nature of the anion secreted is a matter of relative indifference so long as it has the appropriate charge.

Mechanism of the hemopoietic depressant action of whole liver and choline hydrochloride upon polycythemic dogs. JOHN EMERSON DAVIS (introduced by Ray G. Daggs). Department of Pharmacology, University of Vermont, Burlington.

We have shown previously (1938, 1939) that the polycythemias induced in dogs by the oral administration of cobalt or by low atmospheric pressure are depressed by the feeding of whole raw liver or choline hydrochloride.

In an effort to learn more about the mechanism of choline action, we have fed polycythemic dogs 1, pure egg lecithin, one gram daily; 2, betaine hydrochloride, up to 5 grams daily, and 3, choline or liver together with atropine sulfate, the latter in a daily dose of 0.5 mgm. per kilogram of body weight.

Neither lecithin nor betaine affected significantly the polycythemic red cell counts of two dogs, from which we infer that lecithin probably does not yield choline in sufficient quantity within the body to depress hemopoiesis, and that choline does not reduce polycythemia by virtue of its lipotropic properties.

The oral administration of atropine in the dose stated above, however, blocked the hemopoietic depressant action of choline in three polycythemic dogs, as well as the similar action of whole liver feeding in four polycythemic dogs. Polycythemia was produced by cobalt feeding in two of these dogs, by daily exposure to low atmospheric pressure in one, and by both cobalt and low pressure in the fourth dog.

These experiments furnish additional evidence that choline is probably the active constituent of liver concerned in the depression of polycythemia; and that the action probably is a peripheral "muscarine" action of choline.

An assay method for human chorionic gonadotrophin. E. DELFS (introduced by E. B. Astwood). Department of Obstetrics, Johns Hopkins University and Hospital, Baltimore, Md.

A method is presented for the assay of chorionic gonadotrophin in human serum based upon the weight increase of the uterus of the immature rat. Gonadotrophic substance was prepared free of estrogen from pooled serum of 100 pregnant women in the last month of gestation. This was given in graded doses to groups of 21 to 23 day old, 34 to 42 gram female rats. The total dosage was given in 6 subcutaneous injections over a 2 day period and the animals were killed 72 hours after the first injection. By this technique the observed uterine weights plotted against the dose gave a curve which showed direct proportionality between dose and response. This proportionality covered a range 10 times the minimum effective dose. For greater accuracy, only the lower steep portion of the curve between the minimum effective dose and 7 fold increase was used; that region of the curve represented uterine weight increase from 150 per cent to 420 per cent. Observations made on vaginal changes, ovarian weights and gross and microscopic appearance of the ovaries were compared with that curve. Assays using the usual 96 hour tests were also compared with the 72 hour standard curve. The sensitivity to varying dosage and accuracy of the endpoint were found to be greatest in the uterine weight method. A similar curve of dosage-uterine weight response, developed for international standard chorionic gonadotrophin, was found to superimpose on the standard curve, thus making it possible to express assay results directly in international units.

*Studies on the photoelectrically recorded volume pulses of the finger pad of normal and pathological subjects.*¹ JOHN B. DILLON (by invitation) and ALRICK B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

Radial and digital volume pulses simultaneously recorded by photoelectric plethysmographs were compared as to form and time relations. In young and middle-aged adults without apparent sclerosis and with normal

¹ Aided by a grant to A. B. H. from the Council on Pharmacy and Chemistry, American Medical Association.

blood pressures the main deflection of the radial pulse takes between ten and sixteen per cent of the pulse cycle, the main deflection of the digital pulse taking from one to five per cent longer; this difference usually disappears by the time the crest of the rebound (dierotic) wave is reached. The rebound wave uniformly appears higher on the catacrotic limb of the digital pulse than on the radial pulse. There is frequently no relation as to the degree of rebound. The rebound wave tends to climb higher on the catacrotic limb of the digital pulse with age. It may still be present on the radial pulse wave after it has disappeared from the digital pulse in cases of sclerosis. There is an increase up to four times in the delay between the summits of the radial and digital main deflections in cases of sclerosis without hypertension and in cases of hypertension. In several cases of hyper-reactors to the cold pressor test the delay has also been seen, although the resting blood pressure is normal. There is a marked tendency for both radial and digital main deflections to flatten or break towards their apex in cases of hypertension, sclerosis, and in cases of hyper-reactors, although the flattening is seen in the digital pulse first and frequently before there is any disturbance in form on the catacrotic limb. This flattening has not been seen in the pulse waves of individuals with normal blood pressures and giving normal responses to the cold pressor test. Not all hyper-reactors or individuals with elevated blood pressures show disturbances in the time relations and form of the radial and digital volume pulse waves. Constriction of the digital vessels usually does not significantly alter the fundamental form of the normal digital pulse (except for the rebound wave being higher on the catacrotic limb) or the time relations between the radial and digital pulses.

The rôle of the liver in resistance to insulin action. L. B. DOTTI. Department of Physiology and Biochemistry, New York Medical College, Flower and Fifth Avenue Hospitals, New York City. (Read by title.)

The sensitivity of the rabbit to insulin as measured by the time of onset of convulsions, following insulin injection, is related to the state of the liver. After an inanition period of twenty-four hours, rabbits were injected with two units of insulin per kilogram of body weight, and the time of onset of convulsions noted. The injections were given weekly for four weeks. The animals that survived were killed and autopsies performed on all.

The rabbits were divided into two groups:

Group A. In this group of eighteen, were included all the animals with no liver pathology. Of these rabbits, five experienced no convulsions, and in the remainder, sixty-one per cent of the injections resulted in convulsions. The average time of onset of convulsions was 215 minutes.

Group B. In this group of twenty-one rabbits, all the animals showed some liver pathology. All of the rabbits experienced convulsions, and eighty-three per cent of the injections resulted in convulsions. The average time of onset of convulsions was 149 minutes.

These results would indicate that investigations on the responses of rabbits to insulin might be misleading if the possibility of liver pathology is not considered.

The absorption of glycine and histidine from isolated loops of the ileum of urethanized dogs. J. R. DOTY (by invitation) and A. G. EATON. De-

partment of Physiology, Louisiana State University School of Medicine, New Orleans.

Previously reported experiments, using the Cori technique with rats, demonstrated that the basic amino acids were absorbed in proportion to the amount fed. Glycine, on the contrary, appeared to be absorbed at a practically constant rate regardless of concentration. The present study was designed to determine whether this phenomenon, shown by the basic acids, is due to a greatly increased rate of diffusion with rising concentration or to some other factor, such as, the utilization of a greater absorbing area of the small intestine. For this reason we have resorted to closed loops in which the absorbing surface area remains relatively constant. By varying certain conditions we have attempted to obtain further evidence regarding the nature of the processes concerned with the absorption of the amino acids.

No such proportionality between absorption rate of histidine and its concentration within the loop was demonstrated as in the case of the intact rat. Both histidine and glycine, however, are absorbed at somewhat elevated rates as the concentration is increased from very low levels. This is probably indicative of no essential difference in the mechanism of absorption of the two acids.

Monoiodoacetic acid, sodium azide, and phlorhizin, when used within the loop in concentrations not grossly pathological to the mucosa, exert no effect on the rate of absorption. While, as has been suggested, the amino acid mechanism may be somewhat analogous to the "active" process for glucose absorption, it is quite apparent that they differ markedly in their sensitivity to various poisons.

The development of the peripheral pressure pulse of aortic stenosis. PHILIP Dow. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O.

These investigations were designed to show through what mechanisms a stenosis of the aortic valves might produce the tardus quality and the anacrotic phenomena observed in the radial pulse of clinical cases. On the basis of the records of Feil and Katz and the experiments of Katz, Ralli and Cheer, it was predicted that the anacrotic dip would be shown to be developed from the "anacrotic incisura" of the stenotic central pulse.

Dogs were anesthetized with sodium barbital, and in the open chest varying degrees of stenosis were repeatedly produced by a ligature around the root of the aorta, controlled reproducibly by the mechanical screw device used by Katz. Changes in the form of the pulse wave were studied in the aorta-iliac system by the method of Hamilton and Dow, using a long movable cannula up from the femoral artery and a fixed cannula down the carotid to the ascending aorta. Pairs of simultaneous pressure pulses were recorded with Gregg's rubber-membrane high-frequency optical manometers.

The records show that with increasing degrees of stenosis the central pulse assumes both the anacrotic and the tardus qualities. At the same time, the diminution in the violence of the initial ejection of blood reduces the prominence of the long-period natural vibrations usually superimposed on the central pulse as it moves toward the periphery. The peripheral pulse thus becomes a more faithful representation of the central pulse, which in turn now has those characteristics whose source was sought.

Although the axillary-brachial-radial system itself has not been studied, the differences should be only quantitative since the same kind of factors must be operative in both cases.

From the records it appears that the "anacrotic incisura" is not consistently associated with the changes in the peripheral pulse, probably being damped out because of its high frequency.

The effect of lipocaic on cholesterol arteriosclerosis in rabbits. LESTER R. DRAGSTEDT, JOHN VAN PROHASKA (by invitation), DWIGHT E. CLARK (by invitation) and ORMAND C. JULIAN (by invitation). Department of Surgery, The University of Chicago, Chicago, Ill.

The abnormally high incidence of pre-senile arteriosclerosis in diabetes mellitus has been generally recognized and there is some evidence indicating that this may be due to some defect in fat metabolism resulting in sustained hypercholesterolemia. Last year we reported a greater incidence of arteriosclerosis in depancreatized dogs than that normally found in this species. The present report deals with an attempt to prevent the development of experimental cholesterol arteriosclerosis in rabbits by the oral administration of lipocaic. During the course of the work a report appeared by Huber, Broun, and Casey in which they concluded that lipocaic was effective in preventing cholesterol arteriosclerosis and hyperlipemia in rabbits. We found that varying degrees of arteriosclerosis of the aortae could be produced regularly in rabbits in 12 to 20 weeks by feeding from 0.25 to 1.0 gm. of cholesterol dissolved in sunflower seed oil daily. Hypercholesterolemia occurred in the majority of cases but was not constant. Fat-free neutral alcohol extracts of pancreas, containing from $\frac{1}{3}$ to $\frac{2}{3}$ the amount of lipocaic necessary to prevent or cure fatty livers in depancreatized dogs given daily, did not affect appreciably either the incidence of arteriosclerosis or hypercholesterolemia in these animals.

An evaluation of a colorimetric and a biologic method for determining urinary androgens. DELLA G. DRIPS (by invitation) and ARNOLD E. OSTERBERG with the technical assistance of Kathleen Lewis (by invitation), Elaine Limbert (by invitation) and Margaret Casey (by invitation). Section on Obstetrics and Gynecology and the Section on Clinical Biochemistry, The Mayo Clinic, Rochester, Minn.

A biologic method for estimating urinary androgens is discussed in detail. The reactions of the prostate gland and seminal-vesicles of immature castrate rats is standardized with known quantities of crystalline androsterone. The biologic response produced by the urinary androgens is measured in terms of these standards. Reports are given of twenty tests on normal males and forty-six tests on normal females. The output of androgen during three menstrual cycles in one female is considered with reference to the oestrogen output. A discussion is given of forty tests on patients with abnormal genital function. The clinical application of a modification of Oesting's colorimetric determination of urinary androgens is compared with the biologic method considered.

The utilization of ketone bodies by the tissues of animals in ketosis. D. R. DRURY, RICHARD BARNES (by invitation), P. O. GREELEY (by invitation)

and A. Wick (by invitation). University of Southern California, Los Angeles, and Seripps Metabolic Clinic, La Jolla, Calif.

The capacity of normal tissues to oxidize ketone bodies has long been known. Recent work has indicated an ability of tissues of animals in states of ketosis to utilize these substances. It has been our aim to measure the actual ketone body utilization of the tissues in animals showing ketonemia and ketonuria. Ketosis was produced by pancreatectomy, phloridzin, anterior pituitary extract, fasting and ketogenic diets. The utilization was measured by determining arterio-venous differences in the concentrations of these substances. Simultaneous oxygen A-V differences were also carried out. The utilization so determined is of the same order of magnitude as the utilization found by A-V in normal animals injected with B-hydroxy butyric acid at a constant rate.

Site of production of acetone bodies from butyric acid. J. A. DYE and MARSHALL W. STARK (by invitation). Department of Zoology, Cornell University, Ithaca, N. Y. (Read by title.)

Sodium butyrate was injected intravenously into normal, abdominally eviscerated, and depancreatized dogs in amounts sufficient to give, after establishing diffusion equilibrium, optimal utilization concentrations in the blood (150 mgm. per cent, Quastel and Wheatley, *Biochem. J.* **27**: 1753, 1933). All animals were fasting, nembutalized and bilaterally nephrectomized. Blood samples were taken immediately before and at half-hour intervals after the injections. Forty cc. aliquots of the blood filtrate were distilled with dilute potassium dichromate in 14 per cent H_2SO_4 for 30 minutes and the distillate analyzed quantitatively for total acetone bodies (Behre and Benedict, *J. Biol. Chem.* **70**: 487, 1926) and for titratable volatile acids. Due to variable quantities of carbonic acid in the respective distillates, it was necessary to equilibrate the latter with atmospheric air before titrating. Control blood samples contained volatile acids equivalent to about 100 mgm. per cent or more of butyric acid. The net butyric acid was obtained by difference. Control titration values were considerably higher in depancreatized animals.

The results were clear cut and definite. Non-injected control animals maintained constant volatile acid titration values and low basal acetone body concentrations. With the liver intact, injected butyric acid disappeared rapidly and was completely utilized by the end of five hours while the acetone bodies increased to five times their initial level in three hours and then returned to normal about the fifth hour. On the contrary eviscerated preparations showed neither a demonstrable utilization of butyric acid nor a production of acetone bodies. Injected butyric acid was utilized about 20 per cent more slowly by depancreatized animals under similar conditions, but the total acetone body production was about twice that of the normal. If one makes allowance for the higher endogenous fatty acid metabolism of depancreatized animals, it seems apparent that butyric acid metabolism is not abnormal in these animals. The liver is the chief if not the only site of acetone body production from butyric acid. Previous experiments have shown that the utilization of acetone bodies is a function of the extra-hepatic tissues. The metabolism of other fatty acids is being studied.

The heat production and blood and urine constituents after administration of l-histidine to the dog. A. G. EATON¹ and J. R. DOTY (by invitation).

Department of Physiology, Louisiana State University School of Medicine, New Orleans.

The dogs used in these experiments were fasted at least 16 hours before the experiment. Several determinations of basal metabolic rate and blood amino and urea nitrogen values were made in a control period preceding the administration of the histidine. The histidine (8 gm.), as the free base, dissolved in physiological saline at body temperature, was injected either intravenously or intraperitoneally. Histidine monohydrochloride could not be used since it produced an acidosis due to the hydrochloric acid. Blood urea and amino nitrogen levels as well as heat production was determined hourly during a 5 or 6 hour experimental period. Heat production was measured by the Tissot Haldane method, urea by the manometric urease method of Van Slyke, and amino nitrogen by the manometric method of Van Slyke.

There was a prompt and rapid rise of urea nitrogen in the blood. At the end of the period the amino nitrogen level of the blood was approaching the control level. Blood urea nitrogen values were still well elevated at the end of the period. There was a considerable increase in the output of urea in the urine but not enough, however, to account for all of the nitrogen administered. In marked contrast to glycine, to arginine and especially to lysine, previously reported, histidine, even when injected intravenously, was not excreted in large quantities into the urine. In contrast to the single experiment of Rapport and Beard, our results show that histidine produces a definite and prolonged specific dynamic action. The large increases in blood and urine urea nitrogen also indicate that histidine is rapidly metabolized by the dog.

Inhibition of lactation in the rat. ABRAHAM EDELMANN (by invitation) and ROBERT GAUNT. Department of Biology, Washington Square College, New York University. (Read by title.)

In view of certain unsettled questions a study of the lactation inhibiting properties of various hormones was made in 79 rats. Growth rates of litters, reduced to 4 in number, were indices of lactation. In cases quoted here treatment was started at delivery and continued 15 days. A latent period of about 8 days preceded noticeable effect with any treatment. This period could be reduced if injections were begun 3 to 4 days before delivery. Growth of the young of treated mothers was compared to that of 15 litters of similar size from untreated rats.

Chorionic gonadotropin (Follutein—100 RU per day) had no apparent specific effects on the lactation of castrate mothers, contrary to de Jongh's findings in mice. A slight diminution in growth observed with this treatment was probably not a hormone action.

Pregnant mare serum (Gonadin—0.5-1.0 cc. per day) was an effective inhibitor in intact animals, but clearly without effect in ovariectomized rats. Therefore, while a high lactogenic level may inhibit release of gonadotropins (Bates *et al.*), the converse is not true.

Estrone (Amniotin—200 IU per day) inhibited lactation in intact ani-

¹ Aided by a grant from the Committee on Scientific Research of the American Medical Association.

mals, but was without effect in castrates in doses of 300-5,000 IU per day. An inhibition could be obtained in castrate animals if larger doses (1 mg. per day) of estradiol-17-propionate (Follacron) or of diethylstilboestrol were used, but it was distinctly less than obtained from the same doses in intact mothers. Folley and Kon reported similar results with other estrogens. Three mg. of testosterone propionate (Oretone) per day likewise had a slight effect in castrate and a marked effect in intact mothers.

In an attempt to induce in castrate rats an inhibition like that seen in intact ones, 1 mgm. of stilboestrol plus either 3 mgm. of testosterone propionate or 2 mgm. of progesterone (Proluton) were given. Lactation was apparently inhibited more than by the stilboestrol alone, although the differences were not great. Such findings if significant are in accord with the idea that under sex hormone stimulation the ovary secretes supplementary amounts of steroids of which progesterone could be one, although the latter substance by itself is not an inhibitor of lactation (Folley and Kon).

Production of "prothrombin deficiency" in rats by mineral oil and the response to vitamins A, D and K. MARGARET C. ELLIOTT (by invitation), BERTHA ISAACS (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The prothrombin time of 120 normal rats weighing 200 or more grams on a control diet averaged 69.8 ± 0.9 (S.E.) seconds, the range being 43 to 103. Weekly bleeding by cardiac puncture for 4 or 5 weeks did not alter the prothrombin time. When the control diet was fed with 20 per cent by weight of liquid petrolatum to 66 rats, 85 per cent of the rats developed a prothrombin deficiency within 30 days. The median prothrombin time was 199 seconds; the range was 118 to 1800+ seconds. When 11 rats suffering from a prothrombin deficiency (median, 480 sec.) were returned to the control diet the deficiency disappeared in 8.6 days. Ten rats with prothrombin deficiency were given 50,000 I.U. of Vitamin A in oil subcutaneously on a single injection, but no improvement occurred in one week. Eleven deficient rats were given 500 I.U. of activated ergosterol similarly and a definite but not a complete improvement occurred in one week. Eight deficient rats were given 2,000 Almquist units of Vitamin K similarly, and a complete cure of the prothrombin deficiency resulted in one week. In these curative tests the diet containing mineral oil was continued.

Electrocardiogram of athletes with cardiac hypertrophy. M. M. ELLIS and CORNEALIA G. ELLIS (by invitation). Department of Physiology and Pharmacology, Medical School, University of Missouri, Columbia.

Electrocardiographic studies were made on 65 young men, average age 21 years, who at the time were participating in major collegiate athletics and who had histories of strenuous indulgence in such activities for several years. In the cardiographic tests the three standard leads were used with the subject in the reclining position.

From x-ray plates of each heart, evaluated by the Eyster formula, 47 cases (72.3 per cent) of the 65 were found to have definite cardiac hypertrophy. Although the electrocardiographic records of 28 cases were without significant deviations, in the electrocardiograms of 37 subjects (56.9 per cent) of the 65 the QRS complex in lead III (sometimes also in

either lead I or II) was splintered or notched like the QRS configuration usually associated with bundle-branch block, but without the characteristic extension of the QRS time interval occurring in bundle-branch block. The QRS interval for these 37 cases averaged in lead III 0.07 second, ranging from 0.06 to 0.10 second.

Considering the modified QRS complex and the cardiac hypertrophy together, 33 subjects (70.2 per cent) of the 47 showing definite cardiac hypertrophy had the splintered or notched QRS, and 33 (89.2 per cent) of the 37 with splintered or notched QRS had measurable cardiac hypertrophy. In the electrocardiograms of the 33 men displaying the combination of cardiac hypertrophy and modified QRS in lead III, only one case of axis deviation (slight left) was noted and no displacements of the RT segments nor inversion of T-waves were found, consequently the electrocardiographic picture of these athletes was not comparable to that reported for ventricular hypertrophy produced by arterial hypertension. As injuries to specific muscular units of the heart are known to produce characteristic changes in the electrocardiogram the modifications noted here in the electrocardiograms of those athletes with cardiac hypertrophy may have resulted from selective hypertrophy of portions of the ventricular wall brought about by the physiological demands of the strenuous athletic activity.

Growth and reproductive physiology in vitamin B₆ deficiency. GLADYS A. EMERSON (by invitation) and HERBERT M. EVANS. Institute of Experimental Biology, University of California, Berkeley.

Groups of six male rats were placed at weaning on a vitamin B₆ deficient diet adequate in the other members of the vitamin B complex necessary for rats (thiamin, riboflavin and filtrate factors) and in vitamin E. Two control groups received daily except Sundays fifteen micrograms and thirty micrograms respectively of crystalline B₆. Other control groups received 0.5 gram and 1 gram of brewer's yeast daily. Growth ceased in the vitamin B₆ deficient group at approximately the hundredth day of age. Typical acrodynia occurred. The groups receiving the crystalline B₆ grew well though subnormally. Autopsies of all groups occurred at approximately the 150th day. Estrous females were offered at weekly intervals for ten weeks preceding autopsies. No matings were observed in the B₆ deficient group. In this group, though no cases of completely normal testicular tubules were found, all cases showed some normal tubules and sperm in the epididymis. In contrast with these findings the accessory organs of reproduction were gravely reduced in all members of the group, a finding in harmony with the uniformly defective sexual behavior.

*The urinary excretion of steroid compounds I. Normal male subjects.*¹ LEWIS L. ENGEL² (by invitation), GEORGE W. THORN and ROGER A. LEWIS (by invitation). Chemical Division, Department of Medicine, The Johns Hopkins University and Hospital, Baltimore.

After preliminary investigation the following systematic method for the

¹ This study was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

² John D. Archbold Fellow in Medicine.

separation and isolation of steroid compounds from urine was adopted: normal male urine was hydrolyzed with hydrochloric acid and precipitated with sodium benzoate. A crude steroid mixture was obtained after the removal of benzoic acid with alkali. The crude steroid mixture was subjected to alkaline saponification and the neutral fraction separated into ketonic and non-ketonic fractions. Both of these fractions were separated into alcoholic and non-alcoholic fractions. The four fractions obtained were again treated with the ketone and alcohol reagents in order to effect a more complete separation. The alcoholic fractions were treated with digitonin. Thus, the original neutral fraction was separated into six fractions.

To date the following compounds have been isolated from 1000 liters of normal male urine: *trans*-dehydroandrosterone (68 mgm.), from the ketonic alcoholic fraction precipitated by digitonin; cholesterol (58 mgm.), from the alcoholic-non-ketonic fraction precipitated by digitonin; pregnandiol-3(α), 20 (α) (63 mgm.), from the alcoholic-non-ketonic fraction not precipitated by digitonin; and two compounds (as yet unidentified) m.p. 100°C. (5 mgm.) and 181°C. (50 mgm.), from the ketonic-non-alcoholic fraction.

These observations represent preliminary steps in a study of the origin and metabolism of steroid hormones in normal human subjects and in patients with endocrine disorders.

The coronary blood flow in the denervated heart of the trained dog. H. E.

ESSEX, J. F. HERRICK, F. C. MANN and E. J. BALDES. Division of Experimental Medicine, The Mayo Foundation, Rochester, Minn.

Denervations were done according to the technic of Cannon, Lewis and Britton (Am. J. Physiol. 77: 326). After the dogs had recovered completely, a thermostromuhr unit was applied to the circumflex branch of the left coronary artery. Four or five days later observations were made on the response of the coronary blood flow, pulse rate and blood pressure to exercise on a treadmill and to the intravenous administration of certain drugs. Data on a series of such experiments will be presented.

The effect of ergotamine tartrate on paroxysmal tachycardia. EVERETT IDRIS EVANS. Department of Pharmacology, Medical College of Virginia, Richmond.

Although there are many methods used for the treatment of paroxysmal tachycardia (carotid reflex, oculo-cardiac reflex, mecholyl, etc.) it has been observed by the writer that even after all the commonly used methods have been tried, certain patients will not be relieved of their attacks. It had been observed by the writer several years ago that ergotamine tartrate introduced intravenously will provoke vomiting within several minutes. It is very probable that vomiting by this method is induced by stimulation of the medullary centers, including the vagus center.

It was thought that attacks of paroxysmal tachycardia could be stopped if vomiting could be induced by the intravenous injection of ergotamine tartrate. It has been found that intravenous injection of 0.5 mgm. of ergotamine tartrate will induce vomiting regularly in the human subject. Vomiting usually occurs from six to seven minutes following injection.

If doses from 2.5 to 3.0 mgm. are used in the human subject, vomiting is not induced; furthermore, after these larger doses, vomiting cannot be provoked by apomorphine. This is in accordance with earlier findings on the dog (Evans and Koppanyi), where it was shown that small doses stimulate, large doses inhibit, the vomiting center.

In 9 human subjects suffering from acute attacks of paroxysmal tachycardia, vomiting had regularly been provoked by 0.5 mgm. ergotamine tartrate, the tachycardia relieved, the pulse rate returned to normal in every case within about eight minutes. The pulse usually drops to around 55 to 60 beats per minute and slowly returns to the normal rate. No untoward effects have been observed from the use of ergotamine tartrate for this purpose. If morphine had been administered shortly before the ergotamine tartrate was given, vomiting did not occur, nor was the paroxysmal tachycardia relieved.

The author believes that ergotamine tartrate is a useful adjunct in the treatment of paroxysmal tachycardia.

The prevention of muscular dystrophy in suckling young of E-deficient rats by alpha tocopherol and related substances. HERBERT M. EVANS, GLADYS A. EMERSON (by invitation), and OLIVER H. EMERSON. Institute of Experimental Biology, University of California, Berkeley.

Alpha tocopherol prevented the incidence of muscular dystrophy in the young when administered to mothers on the day of parturition or when administered to the young as late as the fifteenth day of age, although a delay to the eighteenth day was ineffective.

The following substances were also given to mothers or young: phytol, 2,2,5,7,8 pentamethyl 6-hydroxy chromane, gamma lactone (Fernholz) and vitamin K₁. Controls received ethyl laurate. At the dose levels employed prophylactic action of these compounds was not demonstrated. Furthermore, 15 mg. of vitamin K₁ did not lead to the birth of living young in females proven sterile through lack of vitamin E.

The relation of the electrical and mechanical events in the dog's heart and the spread of activity in the right auricle and both ventricles. J. A. E. EYSTER, WALTER J. MEEK and HAROLD GOLDBERG (by invitation). Department of Physiology, University of Wisconsin, Madison.

In a previous report it was shown that the onset of contraction in different regions of the ventricle of the turtle occurs at a time when there is maximum flow of electrical current and maximum time rate of change of current in the region. This electrical state coincides with the maximum potential gradient of the unipolar curve and the main peak of the differential curve recorded from the region. The same relationship is shown to hold for the dog's heart in the present communication. This conclusion is derived from a study of contraction curves and unipolar and differential potential-time curves recorded simultaneously from different regions of the auricular and ventricular surfaces.

Previous work on the sequence of involvement in activity of various regions of the heart has been based on an assumed relationship between electrical and mechanical activity not proven experimentally. Since we now know what features of the electrical curves signal the onset of activity, we may study the sequence of involvement in activity by means of elec-

trical records without assuming an unproved relationship. We have done this for the right auricle and both ventricles of the dog using the main peak of the differential potential-time curve as the criterion for onset of local contraction.

The deposition of glycogen with water and potassium in cat livers. W. O. FENN and LORRAINE F. HAEGE (by invitation). The University of Rochester, Rochester, N. Y.

Livers of 20 cats have been analyzed for H_2O , glycogen, fat, nitrogen, chloride and potassium. The glycogen content of the liver was varied by feeding, starvation and phlorhizin poisoning. The results confirm previous data from rats. By graphical analysis it is shown that 1 gram of glycogen is deposited with 1.5 to 1.8 gram of H_2O , 1 gram of lipid with possibly 0.1 cc. of H_2O , and 1 gram of protein with about 3.3 grams of H_2O . Calculating on the basis of the weight of liver minus glycogen and lipid it is found that the water increases as glycogen increases. Likewise the ratio of water to protein is not constant, but the water decreases as the protein increases. This is due to the water associated with glycogen which is at a maximum where the protein is low. In cats the water is about half as great as in rats per gram of glycogen. The absolute amount of extracellular chloride space increases with the glycogen and the calculated concentration of K in the cell H_2O remains relatively constant and independent of the glycogen concentration.

"Capacigraphic" recordings of uterine motility during pregnancy and labor.

CON FENNING (introduced by A. J. Carlson). Department of Obstetrics and Gynecology, University of Chicago and Chicago Lying-in Hospital, Chicago, Ill.

A discussion dealing with a new type of physiological recorder, and presentation and analysis of normal and abnormal recordings (of uterine motility) secured during pregnancy and labor. Uterine motility was studied in primiparae and multiparae beginning at 25 weeks of pregnancy and extending into the 2nd stage of labor. In addition recordings were made during the third stage of labor and simultaneous bagmanometer and capacigraphic studies were made in the pre-partum and 5 to 7 days post-partum uterus.

The effect of daily administration of insulin¹ on growth and reproduction in the white rat. H. WARD FERRILL (introduced by Wm. deB. MacNider).

Department of Physiology, University of North Carolina, Chapel Hill.

With the extensive use of insulin for conditions other than diabetes mellitus in mind a large series of normal white rats have been given insulin (Iletin, Lilly) over a long period of time to determine whether or not it has any effect upon reproduction and growth. The animals were started on daily injections of 20 to 40 Units of insulin per kilo. body weight at the age of 24 days when they were weaned. This procedure was carried on until the females cast their first litter, had suckled the young and same were weaned. Food and water were available for the animals at all times. Complete records were kept for each animal throughout life.

Weight curves were kept on the animals, both males and females. The

¹ We are indebted to Eli Lilly and Co. for the insulin used in this work.

exact age of each female at date of first delivery was recorded, as well as the number and sex of new born; also, the number, if any, of those dead at birth. Controls, injected with 0.9 per cent salt solution, and others with no injections at all were carried in the same way from the same colony.

Five generations of animals have been thus treated and with the number of animals used (exceeding 300) no effect has been found on growth, or on reproduction, either in size of litters, weight at birth or the age of the mother when she cast her first litter.

The various endocrine tissues from these animals are being studied for cellular changes.

The effect of thyroidectomy on serum cholesterol and basal metabolic rate in the rabbit. WALTER FLEISCHMANN and LAWSON WILKINS (introduced by George W. Thorn). Department of Pediatrics, Johns Hopkins University Medical School, Baltimore, Md.

Thyroidectomy in the rabbit is followed by a sharp rise in serum cholesterol varying from 81 to 340 per cent (average 159 per cent) above the pre-operative level. After the first rise, the serum cholesterol fluctuates markedly, finally becoming stabilized after about 12 weeks at a value 21 to 112 per cent (average 58 per cent) above the base level.

Basal metabolism does not show such marked fluctuations but decreases gradually in six weeks to approximately 40 per cent below the pre-operative level. Thyroidectomized rabbits are much more sensitive than normals to a single dose of thyroxin as can be shown by following the changes in basal metabolic rate, serum cholesterol and creatine excretion. Fluctuations in the serum cholesterol of rabbits are so great, that the level of serum cholesterol cannot be used as an indicator of thyroid deficiency in the rabbit. The relations of these findings in rabbits to similar studies in human beings are discussed.

The effect of adrenalectomy on the blood pressure response to posterior pituitary extract (pitressin). J. L. A. FOWLER (by invitation), A. P. W. CLARKE (by invitation) and R. A. CLEGHORN. Department of Medicine, University of Toronto, Toronto, Canada.

The pressor responses of adrenalectomized cats and dogs to sympathetic nerve stimulation and to the intravenous injection of certain drugs has been the subject of several recent reports from this laboratory. In dogs, shortly after adrenalectomy, the rise in blood pressure following the injection of 2 to 4 units of pitressin was found in several instances to be very much less than in controls, not adrenalectomized. It was of interest to determine what effect pitressin would have in dogs whose adrenals had been removed some weeks or months previously. Several such animals have been examined. They were in good health and showed normal blood values at the time of the experiments. Blood pressure was recorded graphically from a femoral artery, aseptically cannulated. Following the injection of the pitressin a contraction of the pulse wave occurred, a slowing in pulse rate and a gradual drop of pressure from the initial level of 100 to 120 mm. Hg to 30 mm. Hg or lower. Over a period of ten minutes this gradually returned to 100 mm. Hg or thereabouts. Electrocardiographic records were obtained simultaneously. Reasons are discussed for the apparent reversal in the pressor effect of pitressin.

*Effects of cigarette smoking on the skin circulation.*¹ F. E. FRANKE and A. B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

The effects of smoking were studied in twenty-one medical students by means of photoelectric plethysmography of the finger, toe, ear and forehead, records from three or four regions being obtained simultaneously. One-half to one hour exposure to the quiet, dark, comfortably warm room ordinarily preceded the test to allow subject to become adapted to the environment. Care was taken to immobilize the regions studied by the use of supports and casts. Prolonged immobilization has disadvantages causing circulatory alterations.

The smoking was not hurried, symptoms seldom resulting. Both smokers and non-smokers were used, the former inhaling if that was customary. Abstinence from smoking for one or more hours preceded the test. One hour control records were obtained in some cases. The blood vessels in the various regions reacted differently. The forehead vessels were relatively unresponsive, usually showing no change. The finger vessels were the most responsive, usually definitely constricting, occasionally very strongly, but in a few subjects no constriction occurred. A subject may increase the vasoconstriction by inhaling the smoke.

Constriction commonly preceded the smoking by 15 seconds to 2 minutes, at the time when the cigarette was brought out and lighted. In some cases this constriction occurred without alteration in respiration and might be considered "psychic."

Any of the cutaneous regions studied may respond to a deep inspiration by vasoconstriction but the forehead is relatively unresponsive, while the finger probably reacts most, with the ear and toe intermediate. We have observed the same order of reactivity of the blood vessels to smoking, suggesting the dependence of the vasoconstriction on deep inspirations.

These may be responsible for the constriction immediately preceding smoking in some instances.

Subjects showing no vasoconstriction just preceding smoking, respond less to smoking.

"Fake smoking" by puffing on unlighted cigarette may cause greater vasoconstriction in a subject than actual smoking.

The hemolytic action of chyle. L. WILLARD FREEMAN (by invitation) and VICTOR JOHNSON. Department of Physiology, University of Chicago, Chicago, Ill.

Samples of lymph obtained from the lacteals and the thoracic ducts of dogs from 3 to 5 or more hours after the ingestion of animal or vegetable fat have been shown to be strongly hemolytic (*Am. J. Physiol.* **124**: 466, 1938). Samples of lymph obtained from leg or cervical lymphatics or from the lacteals and thoracic ducts of fasting dogs or dogs fed on fat-free diets are not hemolytic, indicating that the hemolytic action accompanies the absorption of the products of fat digestion.

That the hemolytic substance is not bile salt is indicated by the fact that placing solutions of bile salts or bile into the intestines does not give hemolytic lymph. Experiments in which free glycerol was placed into the intestines indicated that this substance was not the hemolysin. On

¹ Aided by a grant to A. B. H. from the Council on Pharmacy and Chemistry, American Medical Association.

the other hand, placing free fatty acid into the intestines gave strongly hemolytic lymph. Analyses of lymph for fatty acids and soaps showed a correlation between the quantities of fatty acids and soaps and the extent of hemolysis. The quantities ranged from 1 to 5 mgm. per cubic centimeter.

In vitro experiments employing the above concentrations gave hemolytic actions of approximately the same magnitude, indicating that the quantities of soaps and free fatty acids found in lymph are probably sufficient to account for the hemolysis obtained in vivo.

It is suggested that during rapid absorption of the products of fat digestion, complete resynthesis to neutral fat particles does not occur, leaving free fatty acids or soaps in sufficient amounts to be hemolytic. The absorption of these substances directly into the slowly moving blood of the intestinal capillaries might be harmful to the organism. The transport of these substances by way of the circuitous route of the lymphatics and their eventual discharge into and dilution by the rapidly moving subclavian stream may be of considerable adaptive significance to the organism.

*Effect on arterial blood pressure following release of acute complete bilateral occlusion of the renal artery and vein.*¹ L. FRIEDBERG, M. LANDOWNE and S. ROBBARD (introduced by L. N. Katz). Cardiovascular Dept., Michael Reese Hospital, Chicago, Ill.

Taquini (1) reported that after complete bilateral occlusion of the artery and vein of the dog for a period of 6 hours, subsequent release of the occlusion caused a marked rise in the systemic blood pressure. This has been interpreted to be evidence for the humoral nature of renal hypertension.

We have repeated his work on 19 dogs, anesthetized with nembutal, sodium barbital or chloralosane.

Blood pressure determinations were made by means of the Hamilton needle manometer, the control pressures being taken just before the release of the bilateral occlusion. The blood pressure changes following release of the occlusion varied. In 8 of the 19 experiments a definite rise in both systolic and diastolic blood pressure was observed, while in 11 no clear change in pressure was noted.

The variable blood pressure responses following release of the renal artery and vein are as yet unexplainable. Post-mortem examination of the animals showed congestion and necrosis of the kidney, indicating that during the prolonged absence of blood flow through the kidney, autolytic processes had set in. These processes may result in the production of abnormal products not necessarily present in the partially ischemic kidney. Thus the positive evidence of a pressor effect obtained in our experiments cannot be used as proof that a humoral mechanism is responsible for hypertension following renal ischemia.

(1) TAQUINI, A. C. Rev. Soc. Argent. Biol. 422, 14: 1938.

Gastric secretion as influenced by glucose and by urine extracts. M. H. F. FRIEDMAN (by invitation), R. O. RECKNAGEL (by invitation) and T. L. PATTERSON. Department of Physiology, Wayne University College of Medicine, Detroit, Mich.

¹ Aided by grants from the Libman Fund and the Dazian Foundation.

Evidence that a substance which inhibits gastric secretion may be extracted from the urine of normal individuals has been reported from this laboratory (Sandweiss et al., *Am. J. Digest. Dis.* **6**: 6, 1939; Friedman et al., *Proc. Soc. Exper. Biol. Med.* **41**: 509, 1939). In dogs receiving repeated hourly injections of histamine, the administration of urine extract results in a marked depression of gastric secretion lasting for 3 to 4 hours. An attempt has been made to overcome this gastric secretory inhibition by various means. One of the methods tried has been that of intravenous administration of fluids (plethora). In acute experiments on vagotomized dogs under nembutal anaesthesia, the administration of glucose solutions by vein results in an increase in the rate of gastric secretion stimulated by histamine. This is presumably due to alterations in the osmotic conditions of the body fluids. However, the augmentation in secretory rate is absent if the glucose administration is preceded by urine extract. The mechanism of inhibition of gastric secretion by urine extract remains obscure; it seems probable however that it involves alterations in the permeability of the gastric glands.

Standard operative temperature, a generalized environmental temperature scale, combining the thermal effects of radiant temperature, ambient air temperature and air movement. A. P. GAGGE. John B. Pierce Laboratory of Hygiene, New Haven, Conn.

Standard Operative Temperature, T_O , based on Newton's Law of Cooling, is defined as the equivalent environmental temperature with which a warm body with surface temperature, T_s , exchanges heat at a *standard* cooling rate, K_O ; i.e.,

$$T_O = T_s - (\text{Heat Loss to Environment}) / K_O. \quad (1)$$

Standard Operative Temperature, as defined above is immediately applicable to all types of calorimetry where the Calorie loss from the animal subject is measured without particular attention to its mode of heat transmission to the environment.

In Partitional Calorimetry (C.-E. A. Winslow, L. P. Herrington and A. P. Gagge. *Am. J. Physiol.* **116**: 641, 1936) where the mode of heat loss is of primary interest, the factors, mean radiant wall temperature (T_W), ambient air temperature (T_A) and air movement, V , must be considered. Relative humidity is omitted from the present discussion as its affects only the efficiency of evaporative cooling, a regulative process separately measurable and distinct from the direct heat loss to the environment by radiation and convection. In terms of the three above factors, Standard Operative Temperature is described by the relation,

$$T_O = \frac{K_R}{K_O} [T_W] + \frac{K_C}{K_O} [(\sqrt{V/V_O}) T_A - (\sqrt{V/V_O} - 1) T_s], \quad (2)$$

where K_O represents the sum of K_R , the radiation constant, and K_C , the convection constant for a chosen standard air movement, V_O . The temperature given by (2) describes an imaginary environment of equal wall and air temperatures with standard air movement in which the animal subject, with the observed surface temperature, T_s , would lose the same amount of heat by radiation and convection as in the original environment described by T_W , T_A and V .

By selecting a standard K_0 and its associated air movement, V_0 , it is possible to establish a temperature scale with a sound calorimetric basis to which all physiological processes may be related, provided one measures first the surface temperature of the animal subject and, secondly, either the total heat loss to the environment by direct calorimetry or the individual environmental factors, wall temperature, air temperature and air movement. Use of such a standard temperature scale makes it possible to differentiate between effects of a purely physical nature and those of a physiological nature.

Effect of alcohol on sexual reflexes in dogs. W. HORSLEY GANTT. Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md. (Read by title.)

Five dogs were given by mouth small (0.5 cc. per kgm. body weight), moderate (1 cc. per kgm.), and large (2 cc. per kgm.) doses of ethyl alcohol in 20 per cent solution. After one-half to two hours the effect was measured on the sexual reflexes—the onset of erection, its duration, and the latent period of ejaculation. The effect on animals varied with the dosage as well as with the “constitution.” In general with all the animals there was an increase of latent period of both ejaculation and erection with shortening of the duration of erection. With the small dose the latent period of erection was increased from 41 seconds to 46 seconds, and the duration of erection shortened from 2.9 minutes (control) to 2.5 minutes (alcohol) with no effect on the beginning time of ejaculation. With the moderate dose the latent period of ejaculation was lengthened from 14.5 seconds control to 25 seconds after alcohol, the latent period of erection lengthened from 29 seconds to 39 seconds, and the duration of erection shortened from 7.8 minutes to 5.2 minutes. The large dose generally abolished all the external manifestations of the sexual reflexes.

The effect of alcohol on the sexual (unconditioned) reflexes measured in these experiments, while similar to that formerly seen on the conditioned motor and salivary food reflexes, was much more pronounced than the action of alcohol on the unconditioned food reflexes. Its general action in all cases however was consistently that of a depressant during the probable peak of the alcohol concentration in the blood. The susceptibility of the various dogs was apparently related to their stability: the neurotic animals were affected to a greater degree than the normal.

Conditioned cardio-respiratory changes accompanying conditioned food reflexes. W. HORSLEY GANTT and W. CHRISTIE HOFFMANN (by invitation). Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md.

The purpose of these experiments was to determine 1, visceral measures—emotional components—of the conditioned food reflexes, and 2, whether there are reliable measures of conditioned excitation and conditioned inhibition other than the conventional secretory and motor phenomena in the conditioned food reflex. Experiments were conducted on three dogs ranging in age from one to eleven years: in two of these, “Billy” and “Sechs,” the heart rates accompanying *old* stable conditioned food reflexes elaborated 3 to 6 years previously were measured, and in the third, “Peik,” the cardio-respiratory changes were followed during the period

of *early* conditioned reflex elaboration. During the 10'' action of the conditioned stimulus all the animals showed an increase in heart rate: from 118 control to 131 during the conditioned stimulus (Billy), 85 to 102 (Sechs), 81 to 110 (Peik). (These figures represent the means of 15 to 35 readings.) There was also a slight increase in heart rate accompanying a conditioned inhibition: in Sechs 82 (control) to 88 (conditioned inhibition); in Peik 81 to 91 (means). The heart rate accompanying the unconditioned reflex (the eating of the food) was approximately the same as the heart rate during the action of the conditioned reflex. The respiratory rate was also increased during conditioned excitation (from 12 control to 18 conditioned), but very little during conditioned inhibition. It thus appears that there is an appreciable and constant change in the heart rate during conditioned food excitation, and that a differentiation can be seen in the heart rate between excitation and inhibition as well as the differentiation formerly noted and conventionally measured in secretion and overt muscular movements.¹

*Some observations on suppression of electrical activity of areas 4 and 6.*²

HUGH W. GAROL (introduced by J. G. Dusser de Barenne). Laboratory of Neurophysiology, Yale University, School of Medicine, New Haven, Conn.

In previous papers from this laboratory it was stated that: 1. local strychninization of area 4-s results in a temporary suppression of the electrical activity of area 4; 2. local strychninization of area 6 does not produce such suppression.

It was subsequently found that: 1. this suppression from 4-s not only appears in area 4 but also, a little later, in area 6; 2. immediately in front of areas L. and A. 6 and, therefore, outside the sensory cortex proper, lies an area electrical stimulation of which produces conjugate deviation of the eyes to the opposite side. Local strychninization of this area produces suppression of electrical activity of areas 4 and 6.

The action of insulin on the glycogen content of isolated muscle. CHALMERS L. GEMMILL. Department of Physiology, Johns Hopkins University, School of Medicine, Baltimore, Md.

The experiments reported at the Toronto meeting (Am. Journ. Physiol. 126: 499, 1939) have been repeated and extended. In these experiments the glycogen content of two strips of the same diaphragm (rat) was compared following a three hour period of shaking in Warburg flasks. In the majority of the control experiments 2.0 ml. of phosphate-Ringer solution with 200 mgm. per cent of glucose were placed in each flask. In the insulin experiments, one flask held the same solution as in the control experiments while the other contained in addition insulin. The results of fifteen control experiments gave an average difference of -0.01 per cent glycogen with a deviation of ± 0.14 per cent. When insulin was added in concentrations varying from 24.4 to 0.03 mgm. per cent the average increment was $+0.25$ per cent glycogen with a deviation from $+0.47$ to -0.12 in the individual experiments. The average value of $+0.25$ per cent represents an

¹ The cardi tachometer used in these experiments was constructed for us through the courtesy of Richard Parmenter, Cornell University.

² Aided by a grant from the Fluid Research Funds of the Yale School of Medicine.

increase of 57 per cent over the corresponding average value of glycogen in the control muscle. Amorphous insulin was used in twenty-eight of the experiments and zinc insulinate in eleven.

Additional experiments were made in which muscles were analyzed for initial and final values of glycogen in order to determine if insulin was promoting a synthetic production of glycogen or inhibiting glycolytic activity. Without insulin, there was an average increment in the experiments of 0.18 per cent glycogen during the three hours of shaking, while with insulin the increment was 0.42 per cent in eleven experiments.

Control experiments were carried out with phenylisocyanate-insulin, a preparation which is 95 per cent inactivated. From 19.8 to 0.22 mgm. per cent of this material gave positive results. However, when the concentration was reduced to 0.03 mgm. per cent no effect was obtained. The same amount of amorphous insulin gave a definite increment. Experiments with ovalbumin showed that this protein had no effect on the glycogen content of the rat's diaphragm. Insulin in Ringer-phosphate solution not containing glucose gave negative results. These findings demonstrate that insulin aids in forming glycogen from glucose in an isolated muscle preparation.

Mixing time of T-1824 in the blood. H. GILDER (by invitation), O. H. MÜLLER (by invitation) and R. A. PHILLIPS. Department of Physiology, Cornell University Medical College, New York City.

T-1824, a blue dye much used for blood volume determinations, was injected into a hind-limb vein of dogs anesthetized with nembutal. Five cc. samples of blood were drawn simultaneously from another hind-limb vein, a fore limb vein, a jugular vein and, in some instances, the portal vein. The concentration of the dye in the serum, determined with the aid of a photo-electric colorimeter using appropriate filters, was identical within 5 minutes in all simultaneously drawn samples, frequently much sooner. The concentration of the dye decreased at first rapidly and then more slowly, reaching a constant slope in 20 to 30 minutes. There is a difference of 10 to 20% in the estimated blood volumes when the extrapolation back to injection time is based on the slope of the curve at 5 minutes instead of the slope at 20 minutes.

Rous and co-workers have shown that this dye is found in liver cells and in the peri-capillary, extra-vascular tissues a few minutes after injection. T-1824 is irreversibly reduced in vitro to a practically colorless substance. These facts lend support to the importance of basing *actual* blood volume on the serum concentration obtained at the earliest time that mixing is complete.

An experimental study of the cardiac vibrations giving rise to the first sound.

A. S. GILSON, J. R. SMITH (by invitation) and W. B. KOUNTZ (by invitation). Departments of Physiology and Medicine, Washington University School of Medicine, St. Louis, Mo.

The vibrations set up by beating hearts have been recorded from the chest walls and directly from the hearts of dogs under surgical anesthesia and from the chests of human subjects with normal or with pathological hearts. The recording mechanism was designed to eliminate air conduction and to follow frequencies as low as five per second without loss of sensitivity. The recording of the lower frequency vibrations has not been

found to interfere with the reading of experimental or of clinical records provided the records are of such amplitude as to permit the excursions of higher frequency waves (ca 50 to 100) to be apparent.

Particular attention has been given to the case of the first sound. It is generally true that neither the lower nor the higher "frequency" vibrations show constant successive intervals during a single "sound." Both lower and higher "frequency" waves show changes when the heart is subjected to experimental procedures which change systolic pressure, stroke volume, etc., and may show independent changes in frequency or intensity.

Functioning of the A-V valves is not necessary for production of a first sound. Changes in character of the audible sound heard when a stethoscope funnel is placed in contact with a failing heart may be correlated with changes in recorded wave form.

It seems probable that the first "sound" is developed by various vibrating elements within the heart (valves, myocardial mass, etc.) set into oscillation by the rise of tension in the muscle fibers. Differences between records taken directly from the heart and from the chest wall reveal modifications introduced by the latter.

*The inactivation of thrombin.*¹ ANTHONY J. GLAZKO (by invitation) and JOHN H. FERGUSON. Department of Pharmacology, University of Michigan, Ann Arbor.

The rate of thrombin inactivation was studied under different conditions. Using thrombins of such strength that the inverse relation of concentration to clotting-time held, it was found that the course of the inactivation followed that of a first order reaction. Thrombin treated with acetone, alcohol and ether, and dried at temperatures greater than 60° still exhibited typical progressive inactivation. The phenomenon was most readily demonstrated with *weak thrombins*, since the absolute increase in the clotting-time was greater than with stronger thrombins. The high critical thermal increment for the inactivation process was of the same order as for denaturation reactions. The temperature coefficient at low temperatures was very small, and the thrombins showed negligible inactivation below 15° (c.f. Am. J. Physiol. **123**: 341, 1938). The reaction was at a minimum in the region of pH 5.5. The addition of different types and amounts of thromboplastin to "aged" thrombin preparations had no effect on the rate of inactivation. At low temperatures (less than 15°) the *amount* of thrombin formed from a given prothrombin solution depends upon the amount but not the kind of thromboplastic agent used. At higher temperatures (38°) the slower rate of thrombin formation with cephalin or weaker brain thromboplastin permits the thermally accelerated thrombin destruction to diminish appreciably the final thrombin yield. No specific antithrombic factors were present in these experiments.

Distribution of electrical charges and the relation between unipolar and differential potential-time curves in the frog's gastrocnemius muscle. HAROLD GOLDBERG (by invitation) and J. A. E. EYSTER. Department of Physiology, University of Wisconsin, Madison.

Detailed localization of the electrical sources and sinks developing in the gastrocnemius muscle during contraction initiated by stimulation

¹ Assisted by a grant from the Horace H. Rackham Fund.

through its motor nerve is derived from a study of unipolar curves recorded against a constant reference curve from 20 or more regions along the muscle. The total period of electrical activity is equally divided by 18 time instants and the potentials measured for each instant on all curves. Results are given in the form of potential distribution curves, relative to resting muscle, for each of the 18 instants. The initial electrical state, characterized by the growth and decline of a group of negative charges (sink) near the femoral end, and several groups of positive charges (sources) near the tendon end, exhibits a spatial motion of all charges. Little or no motion of charges occurs in the final electrical state characterized by a reversal of potential from that of the initial state. Simultaneous recordings of unipolar and differential potential-time curves from these various regions shows a correspondence of the peaks of the differential curve and the gradients of the unipolar curve during the initial electrical state, a situation we have shown to exist in heart muscle throughout the QRS period. This relation, however, does not exist during the final electrical state in skeletal muscle. We have concluded from this evidence and from theoretical considerations that the differential curve is the time derivative of the unipolar curve only when the electrical charges responsible for them are in motion.

The effect of alcohol on brain metabolism. WALTER GOLDFARB, KARL M. BOWMAN (by invitation) and JOSEPH WORTIS (by invitation). Psychiatric Division, Bellevue Hospital, New York City.

Numerous investigators have shown that the effect of alcohol in the body is due to its depressant action on the central nervous system. This action has been demonstrated in both physical and mental performance tests. We have investigated the effects of alcohol on the metabolism of the brain in human subjects. The cerebral metabolism of intoxicated patients admitted to the alcoholic ward was estimated from the arterio-venous difference of oxygen, CO_2 , and glucose. The patients were again tested after recovery from the alcoholic bout. In 10 cases we found that the alcohol depressed the oxygen consumption in 6 instances, had no effect in 3 cases, and produced an elevation once.

Antihormone response to injections of crude and purified pregnant mare's serum preparations. ALBERT S. GORDON, IRVING LEVENSTEIN and HARRY A. CHARIPPER (introduced by Robert Gaunt). Department of Biology, Washington Square College, New York University. (Read by title.)

Eight adult Bartonella-free female rats (150-220 grams) were injected daily with 5 R.U. of untreated pregnant mare's serum (veterinary Gonadin,¹ 1 cc. = 50 R.U.). Another group of 8 received daily 5 R.U. injections of a purified pregnant mare's serum preparation (Gonadin,¹ 1 cc. = 350 R.U.). After 50 days of treatment, the rats were sacrificed, the ovaries weighed and the sera of the animals in each of the two groups pooled. The ovaries of the rats injected with purified Gonadin averaged 273 mgm. in weight and were, with the exception of those of one animal, extremely cystic in nature whereas those animals treated with the untreated

¹ Cutter Laboratories, Berkeley, Calif.

serum showed relatively non-follicular ovaries which averaged 89 mgm. in weight.

The ability of the sera to inhibit the simultaneously administered pregnant mare's serum preparations was tested in 25 to 28 day-old female rats. As judged by ovarian weight, both antisera were definitely inhibitory, the serum of the rats injected with the purified Gonadin revealing slightly, but significantly, greater antihormone content than that obtained from the rats injected with the untreated pregnant mare's serum. Precipitin reactions carried out with both antisera were too weak at this time to warrant any definite conclusions.

The rats injected with the untreated pregnant mare's serum for 50 days showed a drop in red cell count from 8.0-8.6 to 6.2-7.3 millions/cu.mm. After 95 days of treatment the count had dropped to 5.2-5.9 millions/cu. mm. The red blood counts of the rats injected with purified Gonadin or Follutein, a purified pregnancy urine extract, remained unaltered during these periods. We are at the present investigating the cause of the anemia developing in the first group of animals and the possibility that it may account, to some extent, for the differences in the antihormone content of the two sets of antisera.

The mechanism of the diuresis produced by acacia in man. ARNOLDUS GOUDSMIT, JR., and MELVIN W. BINGER (introduced by H. C. Bazett). The Mayo Clinic, Rochester, Minn.

In a previous communication (Am. J. Physiol. 126: 505, 1939) evidence was brought forward indicating that the injection of acacia into dogs increases the rate of excretion of chloride with the urine. It was suggested at that time that such a mechanism might play a role in the diuresis produced by acacia in patients suffering from a nephrotic type of edema. No loss of weight is observed after a course of injections of acacia into non-edematous individuals.

The injection of acacia into patients with a nephrotic type of edema increases the volume of the circulating blood plasma, and dilutes its constituents. The dilution of the serum proteins entails a corresponding loss of colloid osmotic pressure per unit of plasma volume which may exceed the one exerted by the acacia introduced, thus resulting in a net loss. Actual determinations of colloid osmotic pressures of the serum before and after completion of a course of injections of acacia have shown it to be increased in only 43 per cent, decreased in 28 per cent, and essentially unchanged in 39 per cent of the cases.

In many instances chloride excretion increases after the administration of acacia to these patients. This increase is sometimes more than proportional to the increase in the excretion of water. The underlying mechanism is not clear but constitutes an obvious reversal of the conditions existing in a variety of hypoproteinemic states where the excretion of chloride and of water appears impeded in the presence of a normal rate of glomerular filtration. Apparently acacia helps in the reduction of an abnormally large rate of tubular reabsorption of chloride and water toward a more normal level. The relative importance of the colloid osmotic pressure of the serum, of the circulating plasma volume and of the kidney in this mechanism of adjustment will be discussed.

The presence of copper in cytochrome oxidase. MARK GRAUBARD (introduced by Gregory Pincus). Physiological Laboratories, Clark University, Worcester, Mass. (Read by title.)

The oxidation of p-phenylenediamine by inorganic copper in phosphate buffer at pH 7.3 is inhibited stoichiometrically by thiourea, salicylaldehyde, potassium ferrocyanide and cyanide. In the presence of cytochrome and cytochrome oxidase instead of copper, the oxidation of p-phenylenediamine is also inhibited by the above compounds. When to such inhibited systems fresh cytochrome substrate or enzyme, respectively, are added, only the mixture receiving fresh enzyme shows renewed activity. This indicates that it is the enzyme which has been inactivated. Our experiments point to the conclusion that cytochrome oxidase possesses copper as its active catalytic metal and that cyanide tends to poison it rather than the iron of cytochrome.

The sugar utilization of hypophysectomized rabbits. P. O. GREELEY (introduced by D. R. Drury). Department of Physiology, University of Southern California School of Medicine, Los Angeles.

Hypophysectomized rabbits develop hypoglycemia after several hours of fasting. The amount of glucose required each hour to maintain the blood sugar at a constant normal level was determined on fasted rabbits 15 hours to 24 days after removal of the pituitary. Sugar utilization was also determined in several fasted hypophysectomized rabbits before and after hepatectomy or evisceration. Glucose was given intravenously either by continuous injection or at hourly intervals. Frequent blood sugar determinations served to guide the rate of injection so as to keep a normal level. In each series there was a high rate of glucose utilization which varied between 300 mg and 700 mg per kilo per hour. This glucose apparently was oxidized as it could not be accounted for by transformation into glycogen or fat or some other intermediary substance by the liver.

*Aortic blood flow curves.*¹ HAROLD D. GREEN, EDMUND F. SCHROEDER (by invitation) and JOHN H. PASCHOLD (by invitation). Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O. (Read by title.)

Estimation of changes of cardiac output and of velocity of ejection are obtained in anesthetized dogs by means of an orifice and differential manometer (similar to—Green and Gregg, *Am. J. Physiol.* **126**: 508, 509, P, 1939; and green, *Ibid.* **126**: 508 P, 1939) which is inserted into the aorta a short distance below the arch. Pressures are recorded simultaneously at the mouth of the aorta and at the orifice.

The pressure at the orifice often reaches a peak earlier than that at the mouth of the aorta but the initial rise, the main peak and the incisura are delayed 0.01 to 0.02 sec.

The flow accelerates abruptly with the rise of pressure at the orifice, reaches a maximum (2,000 to 5,000 cc. per min.—35 to 80 cm. per sec.) coincident with the first pressure peak, declines to a sloping plateau during midsystole and then abruptly drops to zero at the approximate onset of protodiastole. Backflow then occurs, equaling, at the incisura in the pres-

¹ Aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

sure curve recorded at the orifice, often $\frac{1}{3}$ the maximum systolic forward flow. Following this a brief oscillation of flow, forward then backward occurs. Steadily accelerating forward flow is then recorded, reaching a maximum about the middle of diastole and slowing progressively to the end of diastole.

The mean rate of flow is about 500 cc. per min. Increased venous return, epinephrine, aminophylline and asphyxia increase the mean rate of flow, the aortic pulse pressure and the instantaneous rate of flow throughout most of the cycle. Pitressin lowers the mean rate of flow, decreases aortic pulse pressure and delays and diminishes the systolic peak of the flow curve, making it more rounded. Sodium nitrite has little effect on the mean rate of flow but tends to elevate the systolic peak and accentuate and prolong the incisural and early diastolic backflow.

Studies on the linguo-maxillary reflex. R. GREENBERG (invitation) and E. GELLHORN.¹ Department of Physiology, University of Illinois, College of Medicine, Chicago.

In contradistinction to the stimulating effect on respiration and blood pressure, CO₂ causes a decrease in medullary somatic reflexes (linguo-maxillary reflex in the cat). Inhalation of 6 per cent O₂ causes a decrease in the reflex, although respiration and blood pressure increase. The reflex is augmented during hyperventilation. Asphyxia (clamping of trachea) causes a rapid decline in the reflex although respiration and blood pressure increase. The same effects are obtained in animals deprived of their buffer nerves. All effects are reversible on readmission of air. It is inferred that respiratory and vasomotor reflexes show characteristics fundamentally different not only from those of spinal but also of medullary somatic reflexes.

Stimulation of the central end of the vagus, the sciatic and of the depressor nerve (the latter in the rabbit) cause a reversible decrease in the reflex response. The regularity of this phenomenon and its simplicity make it suitable for class room studies on inhibition of reflexes.

The differentiation between vitamin A₁ and A₂ by fluorescence microscopy.

RUVEN GREENBERG and HANS POPPER (introduced by F. T. Jung).

Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago; and the Cook County Graduate School of Medicine, Chicago, Ill.

Vitamin A₂ was demonstrated in fresh water fish (Gilliam, Morton, et al). It differs from Vitamin A₁ in that its maximum absorption bands in the ultra-violet and with antimony-trichloride have longer wave-lengths. Vitamin A₂ has been shown to have biological activity similar to that shown by Vitamin A₁. Rat livers can accumulate Vitamin A₂ when fed fresh water fish liver concentrates (Lederer and Rathman). In the course of fluorescence microscopic studies of vitamin A (Popper) the livers of various fish were studied. In salt water fish a high amount of the characteristic green fluorescence was found in the small fat droplets of epithelial liver cells which fades on ultraviolet irradiation. This distribution is similar to that seen in mammals. In the livers of fresh water fish there was no green fluorescence. However, a yellow-brown fluorescence ap-

¹ Aided by a grant from John and Mary R. Markle Foundation.

peared in similar distribution. The yellow-brown fluorescence faded upon irradiation with ultra-violet light, with the lens system used.

Non-saponifiable fractions of fresh water fish livers (perch) yielded a yellow-brown fluorescence which faded upon ultra-violet irradiation. This yellow-brown fluorescence is in contrast to the greenish fluorescence of vitamin A₁ concentrates (cod liver oil).

The absorption rate of thyroxin pellets implanted subcutaneously in man with myxedema, and in dogs. JAMES A. GREENE and L. E. JANUARY (by invitation). Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City.

The implantation of pellets made from extracts of endocrine glands or from chemical substances has been used recently in the treatment of certain endocrine diseases. In such cases no accurate laboratory procedure has been available to ascertain whether or not the absorption is greater or less than the body requirements. Thyroxin is one endocrine product whose activity can be measured fairly accurately. It was this reason that thyroxin pellets have been implanted in normal and thyroidectomized dogs, and in man. Of the three humans used one had three implantations, another had two, and the third had one. The amount of thyroxin implanted varied from 12.5 to 102 mgm. and it was removed after 14 to 71 days. In the dogs the absorption rate was measured by removing and reweighing the pellets. In man, in addition, the effect upon the basal metabolic rate and clinical manifestations were ascertained. The absorption rate varied from 0 mgm. in 22 days to 6.2 mgm. in 14 days in the thyroidectomized dogs, and from 0 mgm. in 34 days to 3.1 mgm. in 15 days in the normal dog. In man it varied from 0.31 mgm. to 0.44 mgm. per day even in the same individual. This was not sufficient to raise the basal metabolic rate nor to relieve the myxedema. Neither was it enough to maintain the basal metabolic rate nor to prevent the return of myxedema in individuals previously controlled by desiccated thyroid gland.

The body formed a thick fibrinous capsule around the pellets and this was undoubtedly a factor in the variation of rate of absorption.

Experimental intersexuality: the effects of combined androgens and estrogens on prenatal sexual development of the rat. R. R. GREENE and M. W. BURRILL (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The authors have previously demonstrated that the administration of large amounts of androgens to the pregnant rat causes masculinization of the female offspring. These females have well differentiated epididymides, vasa deferentia, seminal vesicles and ejaculatory ducts combined with complete oviducts, uteri and upper vagina. No lower vagina is present, but a male type of urethra with typical male ventral and posterior prostates, coagulating glands, Cowper's glands and a penis are found.

The administration of large amounts of estrogens causes a marked feminization of the male offspring. There is inhibition to complete absence of the epididymides, vasa and seminal vesicles combined with the presence of rudiments of the oviducts and uteri. Prostates are completely absent and an upper vagina is present. Male development of the lower urethra and external genitalia is inhibited. The testes are maintained in the position typical of the female gonads. Nipples are present.

In the present study various doses of androgens and estrogens were administered simultaneously to pregnant rats. Depending on the dosages used, the estrogens have antagonized the masculinizing effects of the androgens on differentiation of the Wolffian ducts and urogenital sinus. Unlike other biological antagonisms between androgens and estrogens, nearly equal or even greater amounts by weight of estrogens must be used to obtain these effects.

Similarly the androgens have antagonized the feminizing effects of the estrogens on the derivatives of the Wolffian ducts and urogenital sinus in the male offspring. This effect has also varied with the relative dosages used.

Uterine hemorrhage in normal, hypophysectomized, and adrenalectomized immature rabbits after estrogen treatment. R. O. GREEP and BERNHARD ZONDEK (introduced by H. B. van Dyke). The Squibb Institute for Medical Research, New Brunswick, N. J. and the Rothschild-Hadassah Hospital, Jerusalem, Palestine.

Uterine bleeding has been observed by one of us (Bernhard Zondek) in normal immature rabbits following intravenous estrogen treatment. These experiments have been repeated with similar results. The possibility that the pituitary gland or the adrenal glands may act as intermediaries in the production of these hemorrhages has been studied in animals in which these glands were removed. Although bleeding has been observed less frequently after hypophysectomy or adrenalectomy than in normal animals, there seems to be no basis for assuming that the action of estrogen is other than direct. Since the bleeding starts several days following the cessation of estrogen treatment and the areas of hemorrhage show histological evidence of regression, it is suggested that the bleeding is associated with involution of the uterus following estrogen stimulation in much the same manner as bleeding occurs in the primate uterus following estrogen withdrawal.

Bleeding occurs from greatly dilated blood vessels in the rabbit uterus. The blood may escape directly into the lumen forming clots that are visible macroscopically (by transillumination), or it may seep into the submucosal tissues in which event bleeding can be established with certainty only in histological preparations. The cells of the uterine epithelium in the areas of hemorrhage are usually greatly flattened and, in small patches, may be loosened or slough away.

Some effects of reflex changes in blood pressure on plasma volume. MAGNUS I. GREGERSEN. Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

A rise in arterial pressure was induced in dogs for periods up to 1 hour by occluding both carotid arteries. The effects on plasma volume were estimated from changes in plasma protein concentration (refractometer) and from deviations in the time-concentration curve of the slowly diffusible blue dye, T-1824 (Gregersen et al., This Journal 1935-39).

Acute experiments were carried out on 8 dogs anesthetized with nembutal. Both vagi were severed low in the neck. Blood pressure was recorded from the femoral artery with a mercury manometer. The average mean arterial pressure before occlusion was 110 mm.; immediately

after 197, and after one hour of occlusion 155. In 4 experiments there was no evidence whatever of a change in plasma volume and in the remaining only questionable evidence of a slight decrease.

Similar tests were made on unanesthetized dogs with modified carotid loops and with the vagi intact. Blood pressure was determined by direct puncture of the femoral artery at the time of clamping the carotids and again when they were released. In 8 experiments on 4 dogs occlusion of the carotids raised the mean arterial pressure from an average normal of 116 to 188; after occlusion for periods of 23 to 56 minutes it was 170 and upon removal of the clamps the pressure fell abruptly to an average of 112. It is important to note that the procedures caused no observable disturbance in the dogs either at the time of the experiment or subsequently. In no instance was any change observed in the plasma volume during the period of elevated blood pressure.

Hematocrit determinations were made in 4 experiments. The average per cent cells before occlusion was 42.0 and during the period of clamping 42.2. It therefore seems unlikely that the reflex vasoconstriction involved the spleen. Furthermore it may be assumed that the sympathetic fibres to the adrenal medulla were quiescent since small doses of adrenaline produce readily measurable rises in the hematocrit. Nevertheless, the pressor effect must be of sympathetic origin inasmuch as carotid occlusion in an unanesthetized totally sympathectomized dog failed to raise the pressure more than 10-15 mm. of Hg.

The relationship between intramyocardial pressure and intraventricular pressure. DONALD E. GREGG and RICHARD W. ECKSTEIN (by invitation).

Department of Medicine, Western Reserve University, Cleveland, O. In the right heart presumably intramyocardial pressure exceeds right ventricular pressure during systole, since its peripheral coronary pressure is greater than right ventricular pressure (Gregg and Dewald, *Am. J. Physiol.* 124: 434, 1938).

In the left heart direct attempts to measure the relative magnitude of the pressures existing in the wall and cavity of the ventricle have been made. The pressure pulse recorded either from a small myocardial pocket of Locke's solution (Gregg and Eckstein, *Am. J. Physiol.* 118: 399, 1936) or from imbedded arterial segments (Johnson and DiPalma, *Am. J. Physiol.* 125: 234, 1939) generally exceeds by a considerable amount the left ventricular pressure simultaneously recorded.

However, these observations do not demonstrate crucially that intramyocardial pressure exceeds left ventricular pressure, since the pressure recorded is at least partially artificially produced by the method used. Our reasons for this belief are: 1. The pressure pulse recorded from an intramyocardial pocket of oil, Locke's solution or blood, although generally considerably greater than intraventricular, increases progressively upon raising the pressure in the pocket. 2. The pressure recorded from a closed vessel segment in the left ventricular cavity may have two to three times the ordinate value of the ventricular pressure. 3. When blood from the aorta flows through an orifice plate meter and then through an artery segment in the left ventricular wall a systolic flow may be present.

The most probable reason for the very large intramyocardial pulse recorded is that it is in part a function of the degree of localized muscle stretch induced by the pocket of fluid or by the imbedded arterial segment.

The conclusion is drawn that direct quantitation of intramyocardial pressure is not yet a reality.

*The effect of sulfanilamide on blood sugar and liver glycogen.*¹ ESTHER M. GREISHEIMER, ROBERTA HAFKESBRING and HULDA MAGALHAES (by invitation). Department of Physiology, Woman's Medical College of Pennsylvania, Philadelphia.

Male rats of 100 to 125 grams, on a diet of Purina Dog Chow Checkers were used in this study. In order to determine the basal levels of blood sugar and liver glycogen, several rats were fasted 18 hours, and then killed by a blow on the head. Blood was collected at this time for sugar determinations (Somogyi-Shaffer micro method). The entire liver was removed immediately and hydrolyzed; an aliquot part was taken for glycogen determinations (modified Pflüger method). The blood sugar averaged 64 mgm. per cent and the liver glycogen 0.28 per cent. Sulfanilamide (1.8 cc. of 1 per cent solution of sulfanilamide powder per 100 grams of rat) given intraperitoneally 3 hours before death did not alter these fasting levels appreciably.

Other rats were fasted 15 hours and then given 3.5 cc. of 10 per cent glucose per 100 grams of rat by stomach tube. These were killed 3 hours later; in this group the blood sugar averaged 81 and the liver glycogen 1.60 per cent. To another group sulfanilamide was given immediately after the glucose. When killed 3 hours later, the blood sugar averaged 89 mgm. per cent and the liver glycogen 2.29 per cent. Thus it seems that both the blood sugar and the liver glycogen are elevated after a single dose of sulfanilamide. Perhaps this indicates a decreased utilization of glucose by the tissues. Studies are in progress with related compounds.

The effect of bile salts on hepatic blood flow. FRED S. GRODINS (by invitation), S. L. OSBORNE (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The relationship of hepatic blood flow to bile secretion has not been extensively investigated. In an attempt to obtain further information on this question, we have studied the changes in hepatic blood flow during the choleresis produced by the intravenous injection of various bile salts. Acute experiments were performed on dogs under sodium pentobarbital anesthesia. Carotid blood pressure was recorded. The common bile duct was cannulated and the cystic duct ligated. Direct current thermistor units were employed to record the rate of blood flow through the hepatic artery, portal vein, and superior mesenteric artery. Extensive preliminary experiments in which the thermoelements were calibrated repeatedly under different conditions both *in vitro* and *in vivo* demonstrated these instruments to be reliable in our hands to within 12 per cent.

The effects of pure sodium 3-7-12 triketocholanate (Decholin) on hepatic arterial flow was studied in 47 experiments on 22 dogs. In 42 instances an increased flow ranging from 10 to 218 per cent and averaging 48 per cent was observed. In 4 cases no change occurred. In two of these, the

¹ Supported by grants from the Penrose Fund of the American Philosophical Society and the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association. The drugs were furnished by the Winthrop Chemical Company, Inc., Department of Medical Research.

choleretic response was slight. In the other two, the animal was totally refractory to any bile salt both in regard to blood flow and bile secretion. The mixed triketocholanates (Ketochol) gave essentially the same results. The unoxidized salt, sodium cholate, gave more variable results (27 experiments, 11 dogs). In approximately $\frac{1}{3}$ of the cases, there was a decrease in flow, in $\frac{1}{3}$ an increase, and in $\frac{1}{3}$ no change.

We have also studied the effects of the pure Na 3-7-12 triketocholinate on the flow in the portal vein and superior mesenteric artery (29 experiments on 13 dogs). The results were similar in each case. In most instances, a decrease in flow was observed. In some cases, an initial decrease was followed by an increase.

Availability of dl-threonine and dl-allothreonine for the formation of carbohydrate in the rat. W. KNOWLTON HALL (by invitation), A. G. EATON¹ and J. ROY DOTY (by invitation). Departments of Biochemistry and Physiology, Louisiana State University School of Medicine, New Orleans.

Two procedures were used in this study. In one case fasting rats were given large amounts of the amino acids and the increases in liver glycogen were determined. In the other series of experiments the effect of these compounds upon acetone body excretion was determined with rats which were being fed butyric acid. The methods used were essentially those of Butts, Dunn and coworkers. In studying glycogen formation rats were fasted 48 hours and then fed 500 mgm. of amino acid every two hours for a period of either 4 or 8 hours. The liver glycogen content was then determined.

The feeding of either dl-threonine or dl-allothreonine resulted in definite increases in liver glycogen. Similarly both substances decreased the ketonuria resulting from the feeding of butyric acid.

Vocal changes in eunuchoidal and castrated men upon administration of male hormone substance. JAMES B. HAMILTON and GILBERT R. HUBERT. Departments of Anatomy and Pediatrics, Yale University School of Medicine, New Haven, Conn. (Read by title.)

The eunuchoidal individual retains a voice high in range and pitch; use of loud tones results in marked elevation of pitch. Men castrated after sexual maturity maintain mature pitch and range. Upon treatment with testosterone propionate, the range and conversational tones become lower, especially in eunuchoids; this precedes detected increase in prominence

PATIENT	STATUS	PRE TREATMENT		DURING TREATMENT	
		Range	Pitch in conversation	Range	Pitch in conversation
1	Eunuchoid	Middle D to high E	F	Low G \flat to middle E \flat	C
2	Eunuchoid	Middle C to high E	G	Low G to middle E \flat	C
3	Castrate	Low G to middle E	B	Low F to middle E \flat	A \flat
4	Castrate	E below middle C to D below high C	G	D below middle C to C above middle C	F

¹ Aided by a grant from the Committee on Scientific Research of the American Medical Association.

of the thyroid cartilage or ossification. Congestion and coarseness of the membranous structures accompany treatment. Withdrawal of therapy permits partial reversion, especially in pitch.

Some reflexogenic components of respiratory rhythm. MARY ALICE HAMILTON (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Varying combinations of sensory stimulation of the vagus, superior laryngeal, and saphenous nerves and chemoceptors were studied. The following are a few examples of the results obtained.

Rhythmic stimulation of the central end of the vagus or superior laryngeal nerve with shocks occurring in blocks at a frequency of 20 to 45 per minute, stopped breathing in the expiratory position. Weaker stimulation produced periodic expirations, frequently compressing the lungs to a sub-expiratory volume, thus showing the central expiratory connections of both nerves.

The addition of a chemical stimulation of chemoceptor endings or of a continuous faradic stimulation of a cutaneo-sensory nerve to the rhythmic stimulation of the vagus or laryngeal nerves frequently interrupted the expiratory pause with an inspiratory contraction. This observation suggests a central inspiratory impingement of the added sensory signals. Vagal or laryngeal stimulation following on this inspiratory act most frequently elicited an expiratory contraction which was followed in turn by a second inspiration of saphenous or chemoceptor origin, etc. A continuous inspiratory drive plus a rhythmical expiratory drive is, therefore, capable of producing rhythmic breathing.

Stimulation of the saphenous nerve frequently decreased the expiratory volume of the lungs below that of normal thus indicating connections with the expiratory half center as well as with the inspiratory half center. Combined synchronized rhythmic stimulation of the laryngeal with the saphenous nerve often increased the depth of both inspiration and expiration over that of laryngeal stimulation alone, indicating that the inspiratory and expiratory connections of the saphenous nerve can be alternately employed to drive the act of breathing.

Expiratory apnea produced by prolonged rhythmic stimulation of the vagus nerve changed at times into a series of inspiratory contractions in phase with stimulation thus indicating the existence of vagal connections with the inspiratory half center. Added chemical stimulation often accentuated this effect.

It is suggested that respiratory rhythm is partly a resultant of central impingement of inspiratory and expiratory signals in which the reciprocating action of the opposing centers directs the prevailing drives from one center to another. Continuous central drive when added to reflexogenic drive is thought to be employed in a similar manner.

*The pattern of the arterial pressure pulse under different conditions.*¹ W. F. HAMILTON. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

The arterial pressure pulse consists of the fundamental curve of systolic filling, diastolic emptying and the periodic vibrations of the main branches of the arterial tree. The main vibrating system which superimposes its

¹ This work was aided by a grant from the Josiah Macy Jr. Foundation.

standing waves upon the filling-emptying pulse is the aortic and its effects can be seen in contour of the pressure pulse in almost any artery. In addition are the standing waves of much shorter period seen in the carotid of the dog, the brachial of man and the leg artery of man. These standing waves seem to be produced 1, if the systolic upstroke of arterial filling is sufficiently rapid in relation to the natural period of the arterial system, and 2, if the incisura is sufficiently pronounced to set up pressure oscillations in the arterial system under consideration.

The standing waves are minimized when the arterial system is of rapid period, (mouse) when systolic filling is very slow or when reflection in the terminal arteries is eliminated by vasodilatation.

That the site of the reflections which produce the arterial standing waves is the arteriolar endings is shown by the injection into one femoral artery of small doses of vasodilator drugs. This brings about local vasodilatation immediately, with the suppression of the first standing wave in the injected artery and no change in the first wave of its mate. When the drug has been carried to the arteries in all parts of the body there is a reduction of diastolic pressure and an elimination of the second wave equally in both arteries. The standing waves on the carotid pulse are eliminated by the same procedure.

The elimination of the standing waves from the femoral artery eliminates the difference between the systolic pressure in femoral and carotid arteries.

We can look upon the pulse contour in "olegemic shock" as seen on the operating table, with the high arterial waves, rapid heart and low mean pressure as the result of vasoconstriction and at "neurogenic shock" with low, flat, slow, pulse contours as the result of overstimulating the depressor receptors in the upper abdomen.

Reflex acceleration of the sympathetically denervated heart in unanesthetized dogs. H. F. HANEY, W. B. YOUNG and A. J. LINDGREN (by invitation). Department of Physiology, University of Oregon Medical School, Portland. (Read by title.)

A selected intravenous dose of acetylcholine in unanesthetized dogs produces a brief fall in blood pressure which initiates compensatory mechanisms resulting in cardiac acceleration. This response is progressively diminished not only by sympathetic denervation of the heart and adrenal demedullation but also by cervical vagotomy.

Sympathetic denervation of the heart was performed by bilateral removal of the stellate and upper 5 thoracic sympathetic ganglia. The vagi were cut in the upper cervical region.

Results: 1. In 23 experiments on 9 normal dogs the average rate increased from a resting level of 105 beats per minute to 250. The maximal acceleration occurred 10 to 15 seconds following the acetylcholine injection, and the maximal rate was reached at 25 seconds.

2. In 2 experiments on 1 dog with the heart denervated except for the left vagus, the rate increased from a basal level of 80 to a maximal rate of 228. The maximal acceleration and maximal rate were attained at the same time as in the normal.

3. In 9 experiments on 4 dogs with the heart completely denervated the rate increased from a basal level of 128, through a maximal acceleration occurring between 15 and 20 seconds, to a maximal rate of 168 at 35 to 40 seconds.

4. In 4 experiments on 2 animals with the heart denervated except for the left vagus, and the adrenal medullae removed, the rate increased from a basal level of 90, through a maximal acceleration which describes a linear curve between 10 and 25 seconds, to a maximal rate of 166 at 25 seconds.

5. In 4 experiments on the same 2 animals after cutting the left vagus, duplication of the experiments described under 4 resulted in a rise from a basal rate of 102, through a maximal acceleration at 15 to 20 seconds, to a maximal rate of 126 at the end of 20 seconds.

It is difficult to explain the above results without postulating the presence in the left vagus nerve of cardio-accelerator fibers which do not originate from the sympathetics. Experiments are being performed in an attempt to further test for the existence of these fibers.

Studies on pain. The analgesic action of morphine and codeine in man.

J. D. HARDY, H. G. WOLFF, and H. GOODELL (by invitation). Russell Sage Institute of Pathology in affiliation with the New York Hospital and Departments of Medicine and Psychiatry, Cornell University Medical College, New York City.

Using the method of measuring the pain thresholds by means of observing the intensity of radiation necessary to evoke a painful sensation in the skin, the pain-threshold raising effect of morphine and codeine have been studied. Morphine sulphate was given intramuscularly in amounts from 0.1 mgm. to 30 mgm., and codeine phosphate in amounts from 15 mgm. to 240 mgm. Observations following injection were made at ten-minute intervals during the period in which analgesia was measurable. The results were in the form of time action curves. The forms of the morphine curves were simple with the maxima appearing at the same time after injection regardless of amount. The total effect was proportional to amount from 0.1 mgm. to 15 mgm. The total effect increased only 17 per cent when the amount was increased from 15 mgm. to 30 mgm. The maximum analgesic effect was proportional to amount between 0.5 mgm. and 10 mgm. For amounts above 15 mgm. little increase in this effect was observed.

The time action curves of codeine were essentially the same form as those of morphine. The total effect of codeine increased in proportion to the amount up to 120 mgm. given at once. The maximum analgesic effect increased in proportion to amount up to 60 mgm.; increased amounts up to 240 mgm. had no further effect.

In the second series of experiments, intense pain was induced by various means for a period of forty minutes. When the painful period immediately preceded the injection of morphine (15 mgm.) or codeine (120 mgm.), the analgesic effect of the agent was almost wiped out. If the agent were given at the beginning of the period of pain 30 per cent of normal analgesia was observed. If the beginning of the pain period were delayed until 40 minutes after the injection, 65 per cent of normal analgesia was realized. Delaying the onset of pain until the maximum effect of the drug had been reached showed that pain then caused little change in the time action curve.

The bioassay of antidiuretic pituitary extracts. KENDRICK HARE. Department of Physiology, Cornell University Medical College, New York City.

The influence of posterior pituitary preparations on the normal urine flow of dogs is slight, but a marked diminution is obtained if the extract is injected during a water diuresis. This antidiuresis can result either from a decrease in glomerular filtration or from an increase in tubular reabsorption of water, or from both. The mechanism of the antidiuretic action of extracts of the posterior lobe of the pituitary was determined from the exogenous creatinine clearances and the creatinine U/P ratios of female dogs with experimental diabetes insipidus. Two or more clearance periods preceded and three or four followed the intravenous injection of the pituitary extract. The periods after the injection did not exceed 10 minutes because it was found that a severe but brief decrease in glomerular filtration may be caused by the extract. The same animal can be used repeatedly to advantage as the response to a known dose is reproduced, and the extreme sensitivity of these polyuric dogs made possible the estimation of as little as one milliunit of pituitrin. The high rate of urine excretion in these dogs does away with the preliminary administration of water to induce a diuresis. However, the administration of creatinine (250 mgm./kg. body weight) by stomach tube and the chemical analyses of plasma and urine add considerably to the time and effort required for an assay. The method allows one to distinguish between the glomerular and tubular action of the antidiuretic substance being assayed.

Spread of excitation in turtle heart as measured by (1) remote and exploring electrodes and (2) two contiguous electrodes. A. SIDNEY HARRIS. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O.

The sequence of apparent arrival of the excitatory process at various portions of the ventral surface of the turtle heart has been studied from records derived by two methods of leading: 1, one remote and one exploring lead, and 2, two leads near together with a total span of one or two millimeters (Differential electrodes record in a similar manner).

The record of potentials from an exploring lead (the other remote) is not a record of activity at a point or even a small area but is influenced by activity a considerable distance away. This is true whether the exploring lead be on heart tissue or a centimeter or two away from the heart in any direction. The potential recorded with the exploring electrode in the bath 1 cm., or less, away from the periphery of the ventricle will have a shape and time of onset similar to that recorded if the lead is placed on the neighboring area of heart muscle. In such a case the initial deflection will be negative or positive depending upon the locus, and consequently the time of excitation of the region as related to excitation of the rest of the heart. Records so made from some portions of the turtle heart show no sharp upstroke of negativity, only the returning limb of a monophasic positive potential. This would be a region among the last to receive the excitation. The earliest areas record negativity only.

Records from leads in the fluid show that the method of leading from two contiguous points records potentials from a much more restricted zone. Potentials arising at distant sources will affect the two electrodes almost equally. The shape will vary with the position of the electrodes relative to the direction of approach of the excitatory wave (Clement; Garten). By rotation of the electrodes the onsets of deflections can be made quite sharp. The time of onset of activity as measured by the two kinds of

leads may show important differences. Because of the more definite localization of origin of potentials affecting the two contiguous electrodes values so derived are considered to be more accurate.

*The ambisexual activity of transandrostenediol.*¹ S. C. HARRIS and R. R. GREENE (introduced by John Gray). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

It is known that both estrogens and androgens cause growth of the uterus in the castrate animal and opening of the vagina in the immature castrate. Only estrogens, however, cause true cornification of the vaginal epithelium, and in this effect they are antagonized by androgens.

It is also known that both estrogens and androgens cause growth of the castrate prostates and seminal vesicles. However only androgens produce histologically normal functional glands. In this effect they are antagonized by estrogens.

It has been reported that transandrostenediol ($\Delta 5$, 3 trans, 17 trans, androstenediol) causes growth of the prostates and seminal vesicles in the castrate mouse or rat (Butenandt, Tschopp) and cornification of the vagina of the castrate mouse (Butenandt, Deansley and Parkes). The findings on the seminal vesicles and prostates were not checked by histological studies and might therefore be due to the "estrogenic" effect of this compound.

Accordingly this substance has been given to castrate male rats (1.0 and 2.5 mgm. daily for 10 days), castrate immature female rats (1.0 mgm. daily for 4 days) and castrate adult female rats (2.0 mgm. daily for 4 days). In the male castrates there was a marked growth of the prostates associated with the histological picture of androgenic stimulation. Premature opening of the vagina occurred in the immature castrate females with cornified smears in both immature and adult castrates. Histological studies showed true cornification of the vaginal epithelium.

Transandrostenediol therefore seems to be truly estrogenic in the female and truly androgenic in the male.

The effect of cortin and of Na factor on the deposition of liver glycogen. FRANK A. HARTMAN and KATHARINE A. BROWNELL (by invitation). Department of Physiology, The Ohio State University, Columbus.

It is well known that liver glycogen may become depleted in adrenal insufficiency. It has further been shown that adrenal extract (Britton and Silvette; Long and Katzin) or corticosterone (Long and Katzin) can raise the liver glycogen of adrenalectomized animals to normal level or above.

In a study of the effects of various adrenal preparations on carbohydrate metabolism we have exposed rats to cold, because differences in response might thus be accentuated. We have been particularly interested in comparing the effects of cortin, which according to our definition has no influence on sodium retention, with those of sodium retaining substances.

The reaction to cold has been studied in normal rats, adrenalectomized rats treated with cortin, adrenalectomized rats treated with Na factor and adrenalectomized rats untreated. Ten or more animals were used in each group.

¹ Supported in part by a grant from the Josiah Macy Jr. Foundation.

Adrenalectomized animals were injected twice daily for 5 days with the respective preparation and on the day of sacrifice, extract was administered every hour for 8 hours. The animals were killed at the end of the ninth hour. They were kept at 27°C. until the last hour (viz., the ninth) during which they were exposed to a temperature of 2°C. During the nine hours of the experimental period they were fasted. The average liver glycogen (expressed as per cent glucose) obtained at the end of 1 hour in the cold was: 2.74 for normal untreated rats, 2.89 for adrenalectomized rats treated with cortin, 1.95 for adrenalectomized rats treated with Na factor and 0.04 for adrenalectomized animals without treatment. The difference in glycogen retention may not be significant because there was no way by which the dosages could be compared. However, the results do show that glycogen retention can be produced by two substances entirely different in respect to their effect on sodium metabolism, one causing sodium retention and the other without effect in this respect.

The spectral sensibility of the long-eared owl. SELIG HECHT and MAURICE HENRI PIRENNE¹ (by invitation). Laboratory of Biophysics, Columbia University, New York City.

The owl's retina contains only rods, and its nocturnal habits have generally been correlated with this fact. Its sensibility distribution in the spectrum as determined by Piper in terms of retinal potentials follows that of other night birds and corresponds to the absorption and bleaching of visual purple in the spectrum.

This whole picture was called into question by the report of Vanderplank that the owl is mainly sensitive to the infrared of the spectrum. However, Vanderplank's work must be in serious error. First, Matthews and Matthews have shown that the far infrared produces no retinal potential in the owl's eye. Second, we have now found that the near infrared also produces no visual effect.

We have further investigated the matter by determining the sensibility distribution of the owl's eye in the spectrum. Our method follows that of Vanderplank in using the iris contraction of the intact animal as a criterion of stimulating action. Exposure to different parts of a continuous spectrum isolated by Wratten and Corning filters show not only that the owl is insensitive to the infrared, but that its sensitivity to red and orange is low compared to its sensitivity to green and blue-green, and that its maximum sensitivity is near 515 m μ , much as is to be expected from the rod nature of its retinal elements. Calling 1.00 the energy at 533 m μ necessary to elicit a minimal contraction of the iris, then the necessary energy at 451 m μ is 3.27; at 490 m μ it is 1.18; at 576 m μ , 3.72; at 631 m μ , 39.5 at 652 m μ , 215.8; and at 683 m μ , 6223.0. Between 750 m μ and 2000 m μ even a relative energy content of 500,000 fails to elicit the slightest contraction in the dilated pupil of the owl.

The iris contracts strongly when the eye is exposed to ultraviolet of 360 m μ . Whether this corresponds to actual vision or only to the fluorescence of the ocular media remains to be decided.

The application of the calcium release theory of stimulation to muscle. L. V. HEILBRUNN. Department of Zoology, University of Pennsylvania, Philadelphia.

¹ Fellow of the Belgian American Educational Foundation.

Muscle protoplasm is extremely sensitive to calcium ion. If an isolated frog muscle fiber is placed in a calcium chloride solution, there is a pronounced shortening, and within 2 or 3 minutes the fiber is reduced to approximately one-fourth of its original length. The uninjured surface of the fiber is relatively impermeable to calcium and the cut ends of the fiber must be exposed to the calcium chloride solution in order that an effect may be produced. In addition to frog muscle various other types of muscle were used. On naked protoplasm generally, calcium ion produces a gelating or clotting effect comparable to the effect it has on blood clotting. As in the case of blood, excess calcium is unfavorable to the reaction in various types of protoplasm, including that of muscle.

Muscle fibers placed in solutions which tend to rob them of calcium ion become non-irritable to electric stimulation by condenser discharges. The irritability is restored on return to solutions containing calcium.

These facts are brought into relation to the calcium release theory of stimulation.

Qualitative changes induced in the gonadotropic complex of the pituitary by androgens. ARTHUR A. HELLBAUM and CARL A. BUNDE (by invitation). Department of Physiology, University of Oklahoma School of Medicine, Oklahoma City.

A study was made of the follicle stimulating (FSH) (gametokinetic) and luteinizing (LH) factors in the pituitaries and blood serum of 1, normal; 2, castrated, and 3, androgen treated male rats. Normal male rat pituitaries contain primarily FSH and only small amounts of LH. Following removal of the gonads, the relationship of the components of the pituitary complex is altered, in that the LH increases in concentration. This factor however, remains stored within the gland and is not liberated into the blood stream. The FSH on the other hand, is released after castration, and its presence may readily be detected in the blood stream.

In contrast to the above, the assay of pituitaries and blood of castrated animals following the administration of androgens, show that androgens release the LH from the pituitary but prevent the liberation of the FSH; the presence of the LH may definitely be demonstrated in the blood of these animals. Daily injection of testosterone propionate prevented the accumulation of LH in adult castrated rat pituitaries; likewise, relatively little LH was found in the pituitaries of rats which had been castrated for 5 months and treated with testosterone propionate for 30 days.

The effect of gelatine on the power of women to perform anaerobic work. F. A. HELLEBRANDT, ROZELL RORK (by invitation) and ELIZABETH BROGDON (by invitation). Department of Physiology, University of Wisconsin, Madison.

Ray, Johnson and Taylor (Proc. Soc. Exper. Biol. and Med. 40: 157, 1939) reported that gelatine invariably increased the work capacity of men but that no appreciable effect was demonstrable in women. The sexual difference, both in muscle power and the effect of gelatine on muscular fatigue, was sufficiently great to warrant repetition of the observations made on women.

The subjects were six females with professional training in physical education. Rapid anaerobic work was performed daily on an electrodynamic brake bicycle ergometer equipped with a graphic voltmeter to record rate of working and speed of pedalling. Duration of exercise was

the independent variable, load and speed being individually standardized. The subject rode to exhaustion. The end-point was inability to maintain the voltage. Five worked at a 290 watt *subject output* and one at 220. Exhaustion was reached in 15 to 91 seconds. This contrasts sharply with the data of Ray *et al.* whose men and women subjects were rapidly fatigued by an *ergometer output* of 60 and 45 watts respectively. Before the end of training the watt minute output of our strongest subject, called "total watts" by Ray *et al.*, exceeded the best gelatine effect in their published data.

Five subjects tolerated 60 grams of gelatine daily. One was given half the dosage. Four experiments were terminated in 8 weeks. Approximately 3 weeks were devoted to preliminary training and 3 to the effects of gelatine. Two served as post-gelatine controls. The watt minute output continued to rise in each series of experiments. The administration of gelatine had no effect on the power to perform anaerobic work. Its withdrawal was without influence. Two subjects trained 8 weeks. Their improvement at that time was essentially the same as that shown finally by the others. The daily administration of gelatine failed to enable them to maintain the high level of performance attained by the rigorous period of training. The gradual fall in the ability to perform work may be due to accruing fatigue.

Studies of central temperature regulation by diathermy electrodes applied to the surface of the hypothalamus of normal unanesthetized dogs. ALLAN HEMINGWAY, THEODORE RASMUSSEN (by invitation) and HOWARD WIKOFF (by invitation). Department of Physiology, University of Minnesota Medical School, Minneapolis.

Under nembutal anesthesia and using a subtemporal approach to the base of the brain, gold diathermy electrodes have been placed on the anterior and posterior hypothalami of dogs. The insulated wire lead attached to each electrode was brought to a subcutaneous position on the skull. About one month after the operation when recovery was complete and temperature regulatory responses were normal, the free end of the wire was brought through the skin and the hypothalamic surface heated by diathermy. Heat production and electrode temperature were measured. It was possible to elevate the hypothalamic temperature with no change in rectal or skin temperatures. Heating the anterior hypothalamus stopped shivering and caused vasodilation of ear vessels but did not induce panting. Heating the posterior hypothalamus caused drowsiness and sleep with a slight reduction in shivering intensity and no vasodilation of peripheral vessels.

Control of coronary blood flow in the heart-lung preparation. J. F. HERRICK, ERICH HAUSNER (by invitation), HIRAM E. ESSEX and E. J. BALDES. The Mayo Foundation, Rochester, Minn.

In the heart-lung preparation the influence of various factors on coronary flow was studied with the thermostromuhr. In agreement with other investigators there was found an intimate dependence of coronary flow on mean arterial blood pressure. Changes in cardiac output had no effect on coronary flow if the arterial pressure and the temperature of the perfusing medium were kept constant. Stimulation of the cardiac branches

of the stellate ganglion of the heart-lung preparation augment coronary flow on the average about 150 per cent. Similar values for coronary flow were obtained in the presence of a constant arterial blood pressure. The increase in coronary flow resulting from stimulation of the sympathetic nerves of the heart-lung preparation is thought to be due principally to a vasodilator action of the sympathetic nerves. When the heart rate was controlled electrically by the method of A. V. Hill (Repetitive stimulation by commutator and condenser) an augmentation of coronary flow was noted with acceleration of the heart rate.

Effect of certain liver diets upon the storage of vitamin A in the rat. R. C. HERRIN and C. A. ENDER (by invitation). Department of Physiology, University of Wisconsin, Madison.

In the course of studies upon nephritis experimentally produced in rats by a diet containing 75 per cent dried liver, it was noted that the vitamin A storage in the livers of these rats was unusually high. In 15 rats the values ranged from 1190 to 2300 blue units (Lovibond) per gram. Diets containing 10 and 30 per cent dried liver fed to young rats for 38 days resulted in much greater liver reserves of vitamin A than the control diets containing casein and lard. Vacuum dried liver purchased from the Wilson Laboratories resulted in liver reserves about like that in the rats fed the control diet. The effect of the liver diet upon the storage of vitamin A in the rat is due to some other factor than its own vitamin A content.

*Photoelectric oscillometry of large, small and smallest arteries in man.*¹ AL-RICK B. HERTZMAN and JOHN B. DILLON (by invitation). Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

The volume pulse has been used in plethysmographic studies as a criterion of blood flow, blood supply and of vascular reactions. It is conveniently and advantageously recorded on a constant base line with the photoelectric plethysmograph and a capacity coupled amplifier. This is equivalent to a slight leak in a mechanical plethysmograph. The technique provides a more legible record of the volume pulse whose recorded amplitude may be given any size desired without inconvenient shifts in the base line.

Comparison of simultaneously recorded (photoelectric) changes in volume and volume pulses in the same vascular area makes possible the distinction of active arterial participation in the plethysmogram. Changes in volume and volume pulse in the finger usually parallel each other in time and magnitude. However, there is often a lag in the volume. Thus, in a response to plunging the opposite hand in ice water, the decrease in digital volume pulse was maximal within five pulse beats, while the decrease in volume occurred eleven beats later. The extent of the changes in volume do not always appear proportional to those in the volume pulse. This may be related to differences in the venous capacity in various subjects and particularly to differences in the venous filling. This is especially true in the forehead skin.

The parallelism between the volume pulse and the volume may fail completely. Thus, in a response of forehead skin to amyl nitrite, there

¹ Aided by a grant to A. B. H. from the Council on Pharmacy and Chemistry, American Medical Association.

was a prolonged delay of nineteen minutes in the return of volume to the pre-administrational level while the volume pulse recovery required only ninety seconds. This difference may be due to prolonged depression of venous tone by the drug.

The volume pulses of larger arteries, as the radial, may be recorded likewise and used to follow reactions there. Thus, throwing a fairly strong light into the dark adapted eye produced constrictions in the finger pad (decreased volume and volume pulse) and decreased volume pulse in the radial artery. The two records showed approximate parallelism except for the smaller waves in the finger which did not appear in the radial artery volume pulse.

Experimental heat exhaustion, a peripheral circulatory failure. FORD K. HICK (by invitation), ROBERT W. KEETON, N. GLICKMAN (by invitation) and M. M. MONTGOMERY (by invitation). Department of Medicine, University of Illinois College of Medicine, Chicago.

In continuing studies of the circulatory adjustments which facilitate heat loss in men exposed to hot environments, we observed that while the subject was in the horizontal position the circulation was adequate. When the subject stood up, dizziness and syncope, evidences of circulatory inadequacy, were observed. Our experiments detail the adjustments of the subjects in the two positions.

Six normal subjects, four male, two female, were exposed to four different environments: cool, 24.1°C. dry bulb and 11.1°C. wet; comfortable, 29.4°C. dry and 13.9°C. wet; hot dry, 37.5°C. dry and 21.1°C. wet; hot wet, 37.5°C. dry and 32.2°C. wet. The air currents in the environment were minimal and the wall temperatures were the same as the dry bulb.

When the subjects were in the horizontal position in the various environments, the changes of the blood pressure, pulse rate, and venous pressures were not remarkable. It was noted, however, that the increase in pulse rate showed a good correlation with elevations in rectal temperature. In the hot conditions, especially when fever was present, the systolic pressure fell greatly on standing and the pulse rate increased to 140 to 160. With continued standing either the changes were progressive until, for example, a blood pressure of 75/65 was reached when syncope appeared, or they reverted toward normal with recovery. In the comfortable and cool environments the blood pressures and pulse rates were not significantly altered on standing.

We believe this syncope constitutes heat exhaustion, in short that experimental heat exhaustion is a peripheral type of circulatory failure, and is occasioned by a disproportion between the circulatory bed and the blood volume immediately available for circulation.

*The effects of alcohol and pentobarbital on metabolism of excised cerebral tissues of adult and infant rats.*¹ H. E. HIMWICH, P. SYKOWSKI (by invitation) and J. F. FAZEKAS (by invitation). Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

The effects of alcohol and pentobarbital on the oxygen consumption of the various parts of adult rat brain (minced, glucose substrate) were exam-

¹ Aided by a grant from the Child Neurology Research (Friedman Foundation).

ined in the Warburg apparatus. This organ was divided in four parts: cortex, cerebellum, medulla, and the remainder, termed brain stem (basal ganglia, thalamus, hypothalamus, and midbrain). Seventy-nine experiments were made with alcohol and 160 with pentobarbital. Comparisons were made with the entire minced brain of infant rats, less than one day old, subjected to the same concentrations of alcohol and pentobarbital in 16 and 44 experiments respectively. Control observations reveal that the relative rates of metabolism of the various parts of adult brain are in the following descending series: cortex, brain stem, cerebellum, and medulla. In the presence of 6 per cent alcohol, the absolute depression occurred in the same order. The percentage depression was approximately the same, 38 per cent, for cortex, cerebellum, and medulla, but was less, 19 per cent, for brain stem. Pentobarbital, 0.012 per cent, caused an absolute depression greatest in the cortex and less in the other parts of the body, which suffer approximately similar decreases of oxygen uptake. On a percentage basis, cortex, cerebellum, and medulla are equally depressed, 38 per cent, but brain stem is less inhibited, 28 per cent. The oxygen consumption of the entire infant brain is much lower than any portion of the adult brain. With alcohol and pentobarbital, both the absolute and the percentage, 21 per cent, depressions are the same. This is less than in the adult brain, calculating on a wet weight basis. These results, like those of Kabat and Dennis, suggest that the infant brain is more resistant to metabolic depression.

Changes in the R.Q. following the administration of glucose to albino rats reported to show hyperfunction of the anterior pituitary. FRED A. HITCHCOCK. Department of Physiology, The Ohio State University, Columbus.

Harned and Cole recently reported that a strain of rats in their laboratory show diabetic type sugar tolerance curves, increased water consumption and urine excretion, and a marked acceleration in rate of growth. These investigators interpret their results as indicating hyperfunction of the anterior pituitary in this strain. It seemed desirable to know what changes would occur in the R.Q. of these animals following the administration of glucose. This question has been investigated with animals obtained from Drs. Harned and Cole (colony A) using as controls animals of the Wistar strain obtained from the same laboratory (colony B). Both animals of colony A and colony B were given by stomach tube 3.5 gms. of glucose per kilo of body weight and then with a minimum of delay placed in the respiration chamber of an open circuit apparatus similar to that described by Carpenter. After a brief wash out period three consecutive thirty minute periods were run on each animal. The first period was begun about 30 minutes after the administration of the glucose. The total ventilation was measured by means of wet test meters and an aliquot of the outgoing air collected and sampled. Air samples were analyzed by means of the Carpenter modification of the Haldane air analysis apparatus, and the O_2 consumption, CO_2 output and R.Q. calculated. Basal tests were run on a few animals.

Averages obtained from experiments on ten hyperpituitary animals and ten controls show lower quotients for the hyperpituitary strain in all periods. This difference is greatest in the first and least in the third period after glucose. Statistical treatment, however, shows that in the

first period only, is the difference more than twice the probable error of the difference; it must be concluded that in the first period after glucose only, do the hyperpituitary animals show an R.Q. which is significantly lower, statistically, than that of the controls. The experimental data seems to justify the conclusion that Harned and Cole's hyperpituitary rats after the administration of glucose show a smaller rise in the R.Q. which occurs more slowly than that of rats of the Wistar strain.

The effect of magnesium on neuromuscular and reflex activity in relation to its concentration in the serum. H. E. HOFF, A. W. WINKLER (by invitation) and P. K. SMITH (by invitation). Laboratory of Physiology, Department of Internal Medicine, and Laboratory of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Conn.¹

An isotonic solution of magnesium sulfate was injected intravenously in 24 cats, anaesthetized with sodium amytal, or decerebrated at the mid-collicular level. Blood samples were taken at intervals to determine the serum magnesium. Neuromuscular twitches disappeared at concentrations of approximately 10 mEq. per l. Neuromuscular tetani produced by electrical stimuli at 20 per sec. disappeared at 15 mEq. per l, and as the rate of tetanic stimulation increased, the level of magnesium necessary for extinction rose. Tetani at 150 to 200 per sec. did not disappear until concentrations of 30 to 40 mEq. per l were reached. Reflexes also disappeared at differing levels. Twitch-like reflexes such as the knee-jerk disappeared at 10 mEq. per l, while fast tetanic reflexes such as the corneal and pinna reflexes were lost at 30 to 35 mEq. per l. The evidence supports the contention that reflex failure is due primarily to neuromuscular block.

Studies of water exchange in dogs with reduced serum electrolyte concentration.

J. H. HOLMES (introduced by M. I. Gregersen). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

Dogs dehydrated by intravenous injection of 50 per cent sucrose (15-26 cc. per kgm.) lose a considerable amount of both sodium and chloride in the urine (av. 51 m.eq. of each). The loss of potassium is usually small (av. 4 m.eq.). When allowed water *ad libitum* the dogs drink amounts equivalent to 70-100 per cent of the weight lost, usually within 1 to 2 hours. At the end of this period serum sodium and chloride are 1 to 15 m.eq. below normal (av. 7 m.eq.), and remain at this level for 48 hours or more. Low serum electrolyte values are not a specific characteristic of the recovery period after sucrose dehydration since the same are observed during the recovery from simple water deprivation. By repeating the sucrose injections once or twice a week and feeding the animals a low salt diet, serum total base and chlorides can be reduced to and maintained at levels 15 to 25 m.eq. below normal. Dogs carried along for 3 weeks on this regime do not lose weight and appear to be in good health.

Such animals offer an opportunity to study the effect of lowered serum

¹ This investigation was aided by grants from Fluid Research Funds, Yale University School of Medicine, Ella Sachs Plotz Fund and The Committee on Therapeutics of the American Medical Association.

electrolyte concentration on mechanisms for regulating water balance. A series of animals with gastric fistulae were allowed to drink *ad libitum* after sucrose dehydration. Sixteen to twenty-four hours later they were given by fistula 40 cc. of water per Kg. In no instance was there a normal diuretic response. Water diuresis was either absent, less than $\frac{1}{2}$ normal, or was delayed $1\frac{1}{2}$ hours or more. In several instances the urine output did not exceed 20 cc. in the 5 hours following administration of 450 to 600 cc. of water. Meanwhile there was a fall in the serum chloride and total base of 5 to 8 per cent which persisted during the 4 to 5 hour observation period. Serum protein and hematocrit changes dropped 10 to 18 per cent. In the control experiments the fall in the hematocrit and serum protein was the same as that of the chloride and total base (5-8 per cent), and all values returned to the control levels within 1 to 3 hours.

Trypsin and insulin action. M. K. HORWITT (introduced by R. W. Gerard). Biochemical Research Laboratory, Elgin State Hospital, Elgin, Ill.

Study of mechanisms which may influence the duration of insulin hypoglycemia indicates that those substances which are anti-tryptic prolong the effect. For example, metals, which inhibit the action of trypsin, also prolong insulin hypoglycemia. Further, insulin exerts an anti-tryptic action, in *in vitro* experiments. The intravenous injection of trypsin not only brings about a hyperglycemia but also inhibits the action of insulin administered simultaneously or after an interval.

Possible influences of trypsin and antitrypsin on insulin action will be discussed.

The effect of desoxycorticosterone, testosterone, and progesterone on the adrenal X zone. EVELYN HOWARD. Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

The adrenal X zone, a differentiation of the cortex, hypertrophies following castration and disappears during pregnancy. Two possibilities suggest themselves regarding the significance of the X zone: it may be a functional specialization of the cortex which responds to certain situations in a different way from the permanent cortex, or, it may be the functional equivalent of the cortex and its hypertrophy or atrophy may reflect quantitative changes in the needs of the organism for cortical hormone. If the latter is the case, one might expect that treatment with desoxycorticosterone would inhibit growth of the X zone, as alleged by Gersh and Grollman.

The effect of injections of desoxycorticosterone was studied in female mice twenty to forty-five days of age. Daily injections of 0.05, 0.1 and 0.5 mgm. of desoxycorticosterone acetate in oil for fourteen days did not prevent normal X zone development. A similar lack of effect was observed with subcutaneous pellets of desoxycorticosterone, which maintain normal growth in young adrenalectomized mice. Testosterone propionate, however, caused almost complete disappearance of the X zone after ten daily injections of 0.5 mgm., and marked degeneration with 0.1 and 0.05 mgm. These testosterone effects are in accord with Deanesly and Parkes' data.

Although larger quantities of desoxycorticosterone might cause inhibition of X zone growth along with inhibition of the entire cortex, it is sig-

nificant that the X zone is more sensitive to testosterone than it is to desoxycorticosterone.

The effect of pregnancy in causing atrophy of the X zone can be imitated by testosterone but not by progesterone, even in daily dose of 5 mgm. This amount of progesterone caused marked uterine enlargement in spayed mice, even without preliminary treatment with estrogen. Estrogens have been reported to stimulate the formation of X zone.

It appears, then, that early X zone disappearance is a reaction somewhat specific to androgen. This accords with other considerations which indicate that the X zone is probably not functionally equivalent to the permanent cortex, and may be more concerned with andromimetic activity.

*The effect of insulin on the electrical activity of nerve.*¹ JOSEPH HUGHES. Institute of the Pennsylvania Hospital, Philadelphia.

The action of insulin on the electrical activity of the cats phrenic nerve has been studied. The nerves used in these experiments were mounted in a chamber through which was passing a mixture of 5 per cent CO₂ and 95 per cent O₂ according to Lehman's technique. The after potentials were recorded on a cathode ray tube.

After obtaining control pictures of the spike and after potentials the nerve was immersed in Krebs-Ringer solution containing zinc free insulin. The pH of this solution was 7.4 to 7.45, the temperature 37.5°C.

Solutions containing 0.002 of a clinical unit of zinc free insulin per cubic centimeter produced a change in the after potentials after one hour of soaking without affecting the spike, conduction rate or refractory period. This effect consisted in a shortening of the negative and deepening of the positive wave. This change in the after potentials has been observed with dilutions as great as 0.0002 clinical unit per cubic centimeter. It is lost at higher dilutions. With dilutions of 0.2 clinical unit per cubic centimeter undulating waves appear in the after potentials. These changes occur at a pH of 7.4. They were abolished by decreasing the CO₂ flow into the nerve chamber. Excitability curves run after the insulin effect was established showed that the irritability of the nerves paralleled the after potential changes. These changes are similar to those reported by Lehman as occurring in nerves in which the calcium has been deionized. Nerves soaked in insulin which had been inactivated by means of NaOH failed to show these after potential changes.

The effect of cortin-like compounds upon the body-weight and work performance of adrenalectomized rats. DWIGHT J. INGLE. George S. Cox Medical Research Institute University of Pennsylvania, Philadelphia.

The substance, 11-desoxy-corticosterone acetate is the most active of the known steroid compounds in its life maintaining capacity. The compound 17-hydroxy-11-dehydro-corticosterone has little life maintenance effect. Our earlier observation has been confirmed that 17-hydroxy-11-dehydro-corticosterone has a greater effect upon the work performance of adrenalectomized rats than does 11-desoxy-corticosterone when the first 24 hours following operation is used as a test period.

In addition, adrenalectomized male rats having an initial body-weight of 180 grams were treated for one week with these two compounds and at

¹ Aided by a grant from the Scottish Rite Masonic fund for Research in Dementia Praecox.

the end of this period their work capacity was studied. The rats treated with 11-desoxy-corticosterone gained in weight even when the doses were small, and although their capacity to work was improved over that of untreated animals, their performance was poor even when as much as 10 mgm. was administered daily. All of the animals treated with 17-hydroxy-11-dehydro-corticosterone lost weight to the same extent as untreated animals, but their work performance was improved above that of the animals treated with 11-desoxy-corticosterone. Similar animals treated with mixtures of the two compounds and with mixtures of their acetates performed more work than animals treated with equal quantities of the single substances.

These findings are in harmony with the hypothesis that there are qualitative differences in the biologic effects of some of the cortin-like compounds.

Respiration of preserved human erythrocytes. R. C. INGRAHAM and A. L. COHN (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago.

The respiration of a suspension of human erythrocytes that had been preserved for periods up to three weeks at $+2^{\circ}\text{C}$. was studied at 37.5° in a Fenn-Thunberg microrespirometer. The samples used were citrated normal human blood preserved for transfusion in the "Blood Bank" of the Cook County Hospital. The cells were resuspended in phosphate buffered Ringer-Loeke solution, and the respiration studied for periods of two hours. It was found that samples not more than 24 hours old respire at a definite and constant rate. Respiration is maintained well over the two hour period of study. Samples preserved one and two weeks do not show any appreciable departure from the behavior of fresh samples. The respiration of blood preserved three weeks shows a marked tendency to drop off during the course of the experiment.

Absorption of carotene from isolated intestinal loops. J. LOGAN IRVIN and JOSEPH KOPALA (introduced by Charles G. Johnston). Laboratory of Surgical Research, School of Medicine, Wayne University, Detroit, Mich.

The absorption of carotene from isolated intestinal loops of dogs has been studied by determinations of the compound in the loop contents with a photoelectric colorimeter before and after the absorption periods. Carotene is not absorbed in analytically significant amounts when the material is placed in the loops in concentrated solutions in cotton seed oil without bile and pancreatic lipase. When the carotene solutions are given with dog, hog, or ox bile, significant amounts of the provitamin are absorbed. Similarly, small amounts are absorbed when carotene in oil is given with pancreatic lipase. However, when the carotene solution is placed in the loop with both bile and pancreatic lipase, much larger amounts are absorbed. For example, 250 micrograms of carotene were absorbed in three hours from an intestinal loop eight inches in length when given with one gram of a crude preparation of pancreatic lipase and one gram of desiccated bile.

The effect of temperature and CO_2 upon the oxygenation of fish blood. LAURENCE IRVING, EDGAR C. BLACK (by invitation) and VIRGINIA SAFFORD (by invitation). Edward Martin Biological Laboratory, Swarthmore, Pa.

The combination of oxygen with the hemoglobin of fish blood is, in many species, extremely sensitive to the presence of CO_2 . In the blood of trout, an increase of pCO_2 from 1 to 10 mm raises the pO_2 for half saturation with oxygen from 18 mm to about 40 mm. The increase in pO_2 required is greater than is shown in the Bohr effect in mammalian blood. Furthermore, the hemoglobin of trout blood is not saturated at $\text{pO}_2 = 150$ mm if CO_2 is present.

We have determined the effect of CO_2 upon oxygen combination with hemoglobin in the blood of three species of trout (Brook, Brown, Rainbow) at temperatures varied after removal between 3° and 35°C .

At 15° the pO_2 necessary for half saturation is 18 mm at $\text{pCO}_2 = 1$ and about 40 mm for $\text{pCO}_2 = 10$ mm. The increase in pO_2 for half saturation is about 1 mm per degree. The CO_2 effect persists up to 35° . Although the blood of the three species is alike at 15° , at other temperatures differences appear and the blood of each species is differently affected by temperature. There is no indication that differences in buffering account for the specific differences in the curves.

At temperatures above 15° the oxygen dissociation curves cease to be hyperbolic and show the "S" shaped curve characteristic of mammalian blood.

The erythrocytes may swell as much as 20 per cent with the change in pCO_2 from 1 to 10 mm.

If the effect of temperature upon blood in the fish is as large as it is *in vitro*, respiratory transport would require different conditions for the three species at any temperature other than 15° .

Variations of muscular tension (action-potentials) in man. EDMUND JACOBSON. Laboratory for Clinical Physiology, Chicago, Ill.

For recording and plotting the magnitudes of action-potentials against protracted intervals of time—minutes or hours, three techniques are now available: 1. The photographic method, employing an amplifier and string galvanometer or oscillograph. 2. The meter method, in which the potentials pass through an a.c. amplifier and an additional circuit in which their average value, after rectification, can be read at each instant on a meter—the Neurovoltmeter or Myovoltmeter. 3. The integrator method, in which the potentials lead from the electrodes (within a frequency range of 20–4000) are amplified, rectified, averaged and integrated over a selected unit of time such as one minute (the Integrating Neuro- or Myovoltmeter). Recordings by each technique are standardized in microvolts and should be accurate to a fraction of a microvolt.

Measurements can be made in platinum-iridium wire electrodes in selected muscle or peripheral nerve in man, reclining or sitting, idle or engaged in a task such as reading. The curves secured from individuals in good health vary considerably depending upon the tissue which contains the electrodes, the character of the electrodes and the contact area, the posture, the occupation, the presence or absence of exciting stimuli, the state of emotion and other factors. "Phlegmatic" types sometimes show relatively high curves if engaged in a task. During so-called "nervous" or "tense" states, relatively high curves are commonly secured from any striated muscular region selected. However, many individuals of "nervous" or "neurotic" type yield curves well within normal limits at times

Measurements were made on four individuals who were not trained to relax but whose daily habit for at least six months previously had been to rest one to two hours per day, generally after the noon meal. In each instance the action-potential record was high, indicating marked failure to relax, in contrast with the results reported on individuals who had received training over a six months period (Am. J. Physiol., 230, 1934).

Effect of castration and androgen treatment on creatine-creatinine excretion in monkeys. JOSEPH W. JAILER (introduced by E. T. Engle). Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.

The purpose of this study was to determine whether any relationship exists between the creatinuria of childhood or castration and the sex hormones. Seven male monkeys were each kept on a creatine-free diet, in individual metabolism cages; creatinine and creatine determinations (Folin) were made on 24-hour samples of urine. The following is the report on 110 control and 156 experimental determinations, together with 26 creatine retention tests.

Two adolescent male monkeys, during a control period of 8 and 18 days, showed a creatinuria which averaged 16 to 18 per cent of the creatinine output. In one, 2 mgm. per day of testosterone propionate for 9 days, and in the other 200 R.U. of PU completely abolished the creatinuria in 72 hours.

Two immature male monkeys, one with a creatinuria of 15 per cent and the other creatine-free (for periods of 15-20 days) were both given creatine retention tests (200 mgm. creatine given intraperitoneally and the amount of creatine excreted in 48 hours determined), and neither could retain more than 31 per cent. During a 9-day period of treatment with 5 mg. testosterone propionate per day, the creatine retention rose to 60 per cent, with a complete abolition of the creatinuria in the animal excreting creatine. Estradiol benzoate temporarily decreases the creatinuria but had no effect upon creatine retention.

Two adult male monkeys, one with an intermittent creatinuria (6 days in a 16-day control period), and the other creatine-free for a control period of 8 days, showed a creatine retention of 62 and 74 per cent respectively. After castration practically a continuous creatinuria set in and the capacity to retain exogenous creatine was decreased. The administration of testosterone propionate abolished the creatinuria and restored the creatine-retention capacity to a level even above that of the control period.

One adolescent male monkey acted atypically. Its creatine excretion was unaffected by castration.

Neither castration, androgen nor estrogen treatment had any effect on the creatinine output.

The isolation of crystalline heparin from the blood of dogs in anaphylactic shock. L. B. JAKES and E. T. WATERS (introduced by C. H. Best).

Department of Physiology, University of Toronto, Toronto, Canada.

Two litres of blood were collected from two big dogs (20 and 18.5 kgm.) sensitized to horse serum and shocked under amytal anaesthesia. Employing, essentially, the method of Charles and Scott, crystalline heparin was isolated from this blood in a good yield, as judged by protamine titra-

tions carried out on samples of the collected blood. This shows, conclusively, that heparin is responsible for the very prolonged coagulation time of blood in canine anaphylaxis. The heparin thus isolated appears to be homogeneous as is crystalline beef heparin, but it is more potent than beef heparin. Using crystalline beef heparin as standard (100 units per mgm.) the canine heparin assayed at about 240 units per mgm. by the Howell method, and at about 130 units per mgm. by the calf blood-thrombin method devised by one of us. It appears to be identical with the heparin we have isolated from dog liver.

Experimentally induced epileptiform discharge and its relationship to local changes in blood flow, pH, and polarization. HERBERT JASPER and THEODORE ERICKSON (introduced by Wilder Penfield). The Montreal Neurological Institute and McGill University, Montreal, Canada.

The electrical activity of the cerebral cortex of the cat has been recorded simultaneously with blood flow (thermocouple), pH (glass electrode), and D. C. voltage changes with non-polarizable electrodes. Blood pressure was also recorded as a control. Epileptiform seizures were induced by metrazol, strychnine and electrical stimulation in animals under light dial or ether anaesthesia and with curare, maintaining constant artificial respiration. After-discharges were induced in the hemisphere from which records were being taken by stimulation of the homologous contralateral region to minimize stimulus artifacts.

Certain of the findings of Dusser de Barenne, McCulloch and Nims (J. cell. comp. Physiol. **10**: 277, 1937) regarding the changes in cortical pH with changes in respiration and changes in electrical activity have been confirmed. A prolonged large amplitude discharge of the cortex was associated with a decrease in pH and an increase in blood flow. The acidity and vascular dilatation were apparently a result of excessive neuronal activity. Strychnine spikes, following the local application of strychnine to the cortex, occurred with no concomitant change in pH or blood flow.

Certain of the apparent initial changes in pH, as recorded with the glass electrode, which appear with the onset of large bursts of electrical activity were complicated by direct current voltage changes of the tissue, as measured between two non-polarizable electrodes on the same area.

Although a definite relationship existed between the epileptic discharge and changes in pH and blood flow, the electrical activity of the cortex varied independent of these changes. The initiation of epileptiform discharge was usually primary to pH and blood flow changes.

Antiserum for dog renin. C. A. JOHNSON (by invitation) and G. E. WAKERLIN. Departments of Physiological Chemistry and Physiology, College of Medicine, University of Illinois, Chicago.

Partially purified extracts of the renal cortex of the dog when injected intravenously or intramuscularly into rabbits evoke precipitins which react with the blood pseudoglobulin present in such extracts and also an antibody which negates the pressor effects of these extracts. This effect on the pressor response to renin was observed in both normal and nephrectomized dogs which were given intravenous injections of antisera previously mixed with renin and kept at 4°C. for 24 hours. Following re-

covery from ether anesthesia for arterial cannulation, mean blood pressure readings were recorded from the femoral artery of the dogs.

In all dogs it was noted that: *a*, the mixing of normal rabbit serum with dog renin has no effect on the pressor response; *b*, the mixing of antiserum with dog renin definitely diminishes or abolishes the pressor response; *c*, the mixing of antiserum with rabbit renin also diminishes the pressor response. The antiserum was somewhat less effective against rabbit renin than dog renin.

The antiserum was found to have no effect on the pressor action of vasopressin.

These observations suggest that the rabbit is able to produce a substance which counteracts the pressor effect of renin—perhaps a specific antibody for renin.

Comparison of human, hog, ox, and dog bile and a study of the effect of desiccation of these biles. CHARLES G. JOHNSTON, CARL E. ANDERSON (by invitation) and J. LOGAN IRVIN (by invitation). Laboratory of Surgical Research, School of Medicine, Wayne University, Detroit, Mich. (Read by title.)

Determinations of inorganic constituents, total bile acids, total conjugated bile acids, glyco- and tauro-conjugated bile acids, cholic acid, desoxycholic acid, cholesterol, bilirubin, phospholipid, free choline, total fatty acids, pH, freezing point depression, surface tension, and viscosity of human and dog hepatic and gall bladder bile and gall bladder bile of the hog and ox have been carried out. In order to obtain figures for the average composition of bile from these types of animals, the determinations have been carried out on pooled specimens from a large number of individuals. Hog bile differs most markedly from the other biles in respect to its qualitative bile acid composition. Human and hog bile have the highest phospholipid, cholesterol, and total fatty acid content. Ox bile has the greatest similarity to human bile in bile acid composition. Desiccation of bile by evaporation under a high vacuum at low temperature produces no changes except loss of carbon dioxide, an increase in pH, and some decomposition of phospholipid. The decomposition of phospholipid can be minimized by the use of fresh, sterile bile and low temperatures.

On the formation of gall stones in the human gall bladder. K. K. JONES and MARIE LORENZ (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Examination of gallbladder bile obtained from nine healthy men after accidental death showed the presence of a putty-like sediment. Analysis of this material revealed a cholesterol content of 9 to 17 per cent, a concentration comparable to that found in mixed cholesterol stones.

On the basis of evidence cited below it would appear that actual formation of a stone does not occur because the presence of sufficient fatty acids and soaps holds the cholesterol in solution, preventing crystallization. First, bile rich in cholesterol and fatty acid when concentrated does not yield cholesterol crystals. Second, an oil saturated with cholesterol, when subjected to saponification, salting out of soap and re-dissolving in water, does not liberate cholesterol at any stage. Further, artificial biles containing fatty acid, bile salt and cholesterol dialyze through

animal membranes without differential separation or change in cholesterol—fatty acid ratio.

These experiments indicate that precipitation of cholesterol in the presence of long-chain fatty acids does not occur in aqueous media or under conditions existing in the gallbladder. It follows that to permit crystallization of cholesterol in the gallbladder these long-chain acids must be modified. Short-chain fatty acids, such as anhydrous butyric acid, dissolve cholesterol but yield it readily in crystalline form upon addition of water. In bile, therefore, when long-chain fatty acids are oxidized to short-chain diffusible components, cholesterol can crystallize.

To date we have found one method by which this conversion may be effected: steam distillation of normal canine or bovine bile yields less than 1 per cent volatile fatty acid. If this same bile undergoes bacterial decomposition the concentration may rise to 40 per cent or more. This suggests that cholesterol stones may result from biliary stasis in which oxidative enzymes from bacteria or leukocytes decompose the long-chain to short-chain fatty acids permitting ready crystallization of cholesterol.

Antagonistic effect of lipocaic on fatty liver produced by ketogenic hormone.

ORMAND C. JULIAN (by invitation), DWIGHT E. CLARK (by invitation) and LESTER R. DRAGSTEDT. Department of Surgery, The University of Chicago, Chicago, Ill.

Suitable extracts of the anterior pituitary have been found to produce a ketonuria (Burn and Ling, 1930), a ketonemia (Anselmino and Hoffman, 1931), and increase in the liver fat (Steppuhn 1934) when injected into rats. A coincident decrease in the amount of depot fat has been found (Best and Campbell, 1936). The administration of choline did not prevent this type of fatty liver.

The purpose of the present study was to observe the effect of lipocaic on fatty livers produced by the administration of ketogenic hormone.

Three groups of young female guinea pigs of approximately uniform weight were used, all animals being fasted for the 24 hours preceding the experiment and during the three day experimental period. The control group was given a daily injection of saline intraperitoneally. The second group was given the ketogenic pituitary extract intraperitoneally each day. The third group was given a suitable preparation of lipocaic in addition to the ketogenic hormone. The animals were killed on the third day of the experiment, 24 hours after the last injection, and their liver weight, liver lipid, and change in body weight determined.

The liver lipid content at the end of the experiment, expressed in milligrams per 100 grams of final body weight, averaged 383 in the control group, 730 in the group receiving ketogenic hormone, and 252 in the group receiving lipocaic and ketogenic hormone.

All animals showed a weight loss. The loss in the control group averaged 51 grams, that of the group receiving ketogenic hormone averaged 61 grams, and that of the group receiving ketogenic hormone and lipocaic averaged 38 grams.

The scoring of dark adaptation tests. FREDERIC T. JUNG and RUVEN GREENBERG (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The use of measurements of dark adaptation in clinical investigations

raises the question of the best method of scoring the test. The data yield a characteristic curve described by Hecht; it should be capable of specification in terms of 5 parameters: 1, the rate of descent during cone adaptation; 2, the limit of cone adaptation; 3, the time of transition; 4, the rate of descent during rod adaptation, and 5, the limit of rod adaptation.

The data on which the following conclusions are based were obtained by 3 sessions with each of 20 subjects. Any given subject had 2 sessions with operator G and 1 session with either N or J. The subjects were medical and graduate students. Coefficients of correlation were then computed, chiefly by the Spearman rank-difference method, between various quantities measured in the tests. Two observations by one operator were found to agree with each other more closely, naturally, than two observations by different operators ($r = 0.61$ for the former and $r = 0.44$ for the latter) despite efforts to avoid variations in procedure. Even these coefficients are rather low, and they represent the *limit* of rod adaptation, item 5 above. Items 2 and 3 are distinctly less susceptible to accurate measurement, and the difficulties regarding 1 and 4 raise doubts as to the utility of rapid tests which have been proposed for the measurement of the *rate* of adaptation.

Within a homogeneous group of people the individual who gave the lowest final threshold on a given day was not likely to rank the same on the following day. Hence the limit of dark adaptation in a given person, as compared with other persons, is determined less by constant or constitutional factors than it is by other variables still uncontrolled.

The influence of age on susceptibility to arrest of the brain circulation. HERMAN KABAT (introduced by M. B. Visscher). Department of Physiology, University of Minnesota, Minneapolis.

We have demonstrated previously that adult dogs surviving periods of brain anemia up to 8 minutes recover consciousness after some time and remain ataxic for periods up to a month or more. Adult dogs surviving 8 or more minutes of cephalic stasis never recover consciousness.

Blood flow to the brain was stopped completely for various periods of time in 50 puppies ranging in age from 1 week to 4 months. These puppies showed a remarkable resistance to arrest of cephalic blood flow and this resistance decreased progressively as the animal grew older.

At the age of 1 week to 10 days puppies which have the brain blood flow stopped for as long as 16 minutes have apparently recovered completely within 18 hours. Such animals have been kept under observation for as long as 7 months and differ in no way from the normal. They grow well, are active, alert, curious, and learn tricks as well as a normal puppy. Puppies at the age of 1 week which survived periods of anemia of 18 or 20 minutes showed evidence of severe brain damage, were unable to nurse and could not be kept alive for longer than 10 days. Thus, once the critical period is exceeded the brain is severely affected by the arrest of its circulation.

During brain anemia for as long as 30 minutes in these young animals spinal shock was never observed although the same procedure always produces spinal shock in the adult animal.

At the age of 1 month, puppies were symptom-free 24 hours after 12 minutes of cephalic stasis. On the other hand at the age of 6 weeks, 12 minutes of complete brain anemia resulted in inability to stand for 3 days

following the procedure and even after 10 days had elapsed the animal was still severely ataxic.

Thus the resistance to brain anemia decreases progressively with age until at about 4 months the animals are as susceptible as are adult dogs. The possible relationship of the greater resistance of the young brain to arrest of its circulation to changes in neuronal metabolism with age is of interest.

*Observations on heart failure in the mammalian heart*¹. L. N. KATZ, K. JOCHIM (by invitation) and E. LINDNER (by invitation). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill. (Read by title.)

Recently the method of determining oxygen consumption, as used by Katz and Mendlowitz (Am. J. Physiol. **122**: 262, 1938), was criticized by Moe and Visscher (Am. J. Physiol. **125**: 461, 1939) on the basis that the coronary sinus blood is not a true sample of the mixed venous blood of the heart. Therefore, we have repeated the experiments using a closed-circuit heart-lung preparation in which a blood reservoir was employed to keep the output and work of the heart constant. The oxygen consumption in these experiments was determined from the product of the coronary flow (difference between pulmonary artery and aortic blood flows) and the A-V oxygen difference (determined simultaneously) between the aorta and pulmonary artery. Calculations show that this actually measures the oxygen consumption of the heart except for that lost in the small amount of blood (less than 10 per cent) which drains into the left heart.

These recent experiments showed that during spontaneous heart failure, cardiac work being constant, the primary change was a decrease in oxygen consumption, the mechanical efficiency remaining practically unchanged. The criticism of Moe and Visscher, therefore, appears to be unwarranted.

These experiments support our previous observations in indicating that the primary mechanism of heart failure is a decrease in energy release and not a decrease in the efficiency with which this energy is converted to work.

*Observations on the potentials established by injury to the heart*². L. N. KATZ, H. SUGARMAN (by invitation), A. SANDERS (by invitation) and K. JOCHIM (by invitation). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

In these experiments we were able to confirm the observations of Eyster, Meek and their collaborators (Am. J. Physiol. **124**: 717, 1938) that an injury to the mammalian heart causes a relative negative potential in this region during inactivity of the heart and relative positivity during the period of activity of the heart. The development and disappearance of these two potentials in the course of the experiment were parallel. The injury in our experiments was produced with the electrode previously described by Jochim, Katz and Mayne (Am. J. Physiol. **111**: 177, 1935). These observations do not contradict the previous experiments from this laboratory concerning the variation in duration of the monophasic action potential.

¹ Aided by the A. D. Nast Fund for Cardiac Research and a grant from the Committee on Scientific Research of the American Medical Association.

² Aided by the A. D. Nast Fund for Cardiac Research.

In a second series of experiments, the potential changes in a series of spots inside and outside a region injured with 95 per cent alcohol were studied by obtaining unipolar electrograms in successive periods following the injury. It was found that this produced: 1. Permanent alterations in the QRS configuration with superimposed temporary changes, both attributable to alterations in the pathway of impulse spread.

2. Injury potentials confined to the region of injury and gradually disappearing; explained in the same way as in the first series.

3. The later development of smaller injury potentials at the periphery of the original injured region; attributed to lymphatic spread of the noxious material from the original injured region.

4. The development of a typical coronary T wave of negative potential, starting in the outermost regions surrounding the original injured area and tending to come closer to the original injured region in successive records. This suggests that the coronary T wave is due to a temporary lag of the repolarization process in the area surrounding the original injury.

We have shown in these experiments that the time and the site at which this T wave potential occurs are different from those which give rise to the injury potential.

Responses of the veins in the extremities. KURT H. KATZENSTEIN (by invitation) and DAVID I. ABRAMSON. The May Institute for Medical Research of the Jewish Hospital, Cincinnati, O.

Limb volume changes and blood flow in the hand, forearm and leg were studied in a series of subjects in different age groups by means of the plethysmographic method.

In young subjects, spontaneous variations in limb volume were consistently observed in the hand and occasionally in the other sites. Excessive heat or cold markedly reduced the magnitude of these changes or eliminated them entirely. In older subjects, spontaneous hand volume variations were either absent or of small magnitude. In some instances, however, during such states as sleep, they became quite prominent.

Evidence was obtained which indicated that these variations in hand volume were principally due to alterations in venous tonus. Although there was an associated change in arteriolar tonus, this factor appeared to play only a slight rôle in the observed limb volume alterations.

These findings are in accord with the view that in the extremities, particularly the hand, the venous bed (i.e., veins, venules and capillaries) are under central nervous control, but can act independently of the arterioles. In view of this, changes in the volume of an extremity cannot be considered to signify changes in arterial inflow alone.

The excretion of potassium by the kidney. NORMAN M. KEITH and ARNOLD E. OSTERBERG. Division of Medicine and Section on Biochemistry, The Mayo Clinic, Rochester, Minn.

After the ingestion of potassium bicarbonate there is an increase in the urinary excretion of potassium. The clearance of potassium rises to a maximum and is followed by a decrease. Simultaneous analyses of the urinary potassium and anions indicate that most of this base is combined with the chloride and bicarbonate and the excretion of phosphate is definitely diminished.

The renal clearance of potassium when compared to that of inulin is

distinctly low. In the normal individual who has been without food for the previous twelve hours it approximates one-tenth that of inulin. With the ingestion of a considerable amount of the bicarbonate, chloride, or nitrate, the potassium clearance rose to one-half that of inulin. Clearance calculated from the experiment of M. Norn, *Skandinavian Archives of Physiologic*, **55**: 215, 1929 showed that following a somewhat larger dose, 12 grams of potassium chloride (6.3 grams potassium) the clearance of potassium may equal that of inulin. These results suggest that if potassium is readily filtered by the glomeruli then it undergoes variable tubular reabsorption.

Studies of patients with severe chronic renal insufficiency indicate that in some cases there is no increase in the concentration of serum potassium and that the kidney retains the function of excreting potassium.

Rage readily elicitable in a dog with descending hypothalamic fibers severed.

A. D. KELLER. University of Alabama. (Motion picture demonstration.)

The dog to be exhibited had the fibers exiting from the dorso-caudal aspect of the hypothalamus severed by a circular cut made with a small rigid probe (milliner's needle). The criterion used, aside from operative visibility, for complete severance of the descending hypothalamic fibers is that the animal exhibits maximal inability to maintain a constant rectal temperature in a cool environment as calibrated against a chronic low midbrain dog. The rectal temperature holds 6°C above the environmental temperature in the *post absorptive state*. Animals having remnants of either posterior or dorsal hypothalamic tissue are able to maintain a rectal temperature higher than this above the environmental temperature. Except for possible infarcts the infringement upon the thalamus can not be extensive.

The dog in its present chronic state (7 weeks) shows a considerable impairment of its mental faculties as judged by his apparent lack of interest in, or awareness of, its environment. When handled gently the dog shows no noteworthy emotional reactions. When handled the least bit roughly typical rage results. Once rage has been elicited the dog remains markedly hypersensitive (pseudo-affective) until an appropriate period of rest supervenes. "Local sign" is retained as shown by snapping and biting at an object touching the body at any point.

This dog re-emphasizes the conclusion drawn from a previous study that a typical rage response is not dependent upon an out flow of fibers from the hypothalamus. In addition the contention that the posterior hypothalamus is essential for a full blown rage reaction is questioned. My primary purpose in exhibiting the animal is to obtain opinions regarding this specific point.

In the event the dog does not survive until the time of the meeting she will be demonstrated by motion pictures.

Adrenal atrophy following hypophysectomy procedures. A. D. KELLER and J. M. BRUHN. University of Alabama.

A pronounced lack of atrophy of the adrenals in the dog following attempted total hypophysectomy plus a rather considerable involvement of the ventral hypothalamus has been encountered. On the other hand

marked atrophy has consistently resulted following ordinary hypophysectomy as well as in instances where it was attempted to attain a total hypophysectomy with little or no infringement upon the hypothalamus.

The failure of the adrenals to atrophy following a drastic hypophysectomy procedure is of sufficient interest to report although the explanation is as yet uncertain. The question arises as to whether we are dealing with occasional accessory (or remnants of) hypophysial tissue, which in our present small series of animals has occurred coincidentally with hypothalamic involvement, or with a true hypothalamic factor. If the former is the case we will eventually encounter a failure of the glands to atrophy in instances of ordinary hypophysectomy, and atrophy following hypophysectomy plus hypothalamic involvement. In this connection we have found that rather sizeable remnants, macroscopically intact, of pars anterior left in relation with the posterior lobe artery following ordinary hypophysectomy has failed to protect the adrenals from atrophy. One would expect however that such remnants might be functional only infrequently. Again the sparing of the adrenals has been selective in that metabolism was reduced (thyroid) as greatly as in ordinary hypophysectomy. If a hypothalamic factor is concerned the failure of atrophy could well be explained by postulating that hypothalamic tissue exerts a *contra-adrenotropic* action which normally is masked by the adrenotropic principle.

Associated with the failure of the adrenals to atrophy has been: 1, an insulin sensitivity near the normal; 2, a corresponding susceptibility to pancreatic diabetes, and 3, a moderate diabetes insipidus. Following atrophy of the glands insulin sensitivity has been increased from 20 to 80 times, the tests being made after the preparations were long chronic. Mild diabetes insipidus has occurred also in the presence of adrenal atrophy. Sexual arrest and obesity have been common to all three operative procedures.

The valvular efficiency in mitral and aortic insufficiency. ANCEL KEYS, H. L. FRIEDEL (by invitation), L. H. GARLAND (by invitation) and M. F. MADRAZO (by invitation). Laboratory of Physiological Hygiene and the Department of Radiology, University of Minnesota, Minneapolis; The Stanford University Radiology Service, San Francisco Hospital, San Francisco; and the Hospital de Ferrocarril de Mexico, Mexico, D. F.

The procedure of simultaneous roentgenkymography and acetylene re-breathing (Keys and Friedell, *Am. J. Physiol.* **126**: 741) for measurement of net stroke output and volume change from diastole to systole was applied in 3 different laboratories to cases of aortic and mitral insufficiency and to cardiac normals. Valvular efficiency was calculated:

$$E_v = \frac{\text{acetylene stroke volume}}{\text{roentgenkymogram stroke volume}} \times 100$$

In 54 studies on normals E_v averaged 105 per cent, minimum 90 per cent, with only 5 cases below 95 per cent. In 18 cases diagnosed "severe regurgitation," E_v ranged from 39 to 64 per cent, average 52.1 per cent, $\sigma = \pm 6.63$. In 16 cases of "moderate" regurgitation E_v ranged from 58 to 78 per cent, average 69.7 per cent, $\sigma = \pm 7.24$. In 11 cases representing the "slightest regurgitation definitely detectable," E_v ranged from

82 to 92 per cent, average 86.9 per cent, $\sigma = \pm 3.09$. All diagnoses were made by independent clinicians.

Ten cases of "compensated" aortic insufficiency, uncomplicated by hypertension, stenosis or mitral lesions, were selected. Systolic pressures ranged from 118 to 150, mean 134.7, $\sigma = \pm 11.34$, pulse pressures 48 to 118, mean 74.6, $\sigma = \pm 23.92$. Calculated E_v ranged from 42 to 84 per cent, mean 69.6 per cent, $\sigma = \pm 13.14$. The coefficient of correlation between pulse pressure and E_v was -0.887 . In these cases the net (acetylene) stroke per m^2 of body surface ranged from 20 to 38 cc., mean 28.0, $\sigma = \pm 5.10$. In 24 cardiac normals the acetylene stroke ranged from 25 to 45 cc., mean 33.9, $\sigma = \pm 6.24$. All studies were made in rest, sitting, under standard environmental and physiological conditions.

Sleepiness and diplopia. N. KLEITMAN and J. SCHREIDER (by invitation).

Department of Physiology, University of Chicago, Chicago, Ill.

It is well known that the diplopia of extreme sleepiness may become so annoying as to discourage vision and lead to a closure of the eyes which makes it all the harder to maintain wakefulness. Is the binocular imbalance due to fatigue of the eye-muscles that are in constant use during wakefulness? To answer that question we determined the variation in the ability of several subjects, remaining awake overnight, to fixate their eyes on a point 12 inches distant, for five minute periods, by photographically recording the eye positions by means of an American Optical Company Ophthalmograph. It appears that the diplopia of sleepiness is not due primarily to muscular fatigue, but rather that binocular imbalance is caused by, or is a component of, sleepiness. Thus, during the night diplopia becomes more and more marked and reaches its greatest extent in the early hours of the morning, when the sleepiness is extreme, but later in the day (after a sleepless night), as the individual becomes less sleepy, good binocular fixation is progressively restored. Likewise, if an individual passes a whole night without becoming sleepy (spontaneously or after the use of benzedrine), he shows no evidence of binocular imbalance.

The determination of V-factor (coenzyme) in blood. HENRY I. KOHN (introduced by G. S. Eadie). Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.

Our method for assaying the nicotinic acid-containing coenzymes in blood has been questioned by Ballif, Lwoff, Querido and Ornstein (Compt. Rend. Soc. Biol. **131**: 903, 1939) because no recoveries have been published. The procedure (Biochem. J. **32**: 2075, 1938; J. Clin. Investigation **18**: 585, 1939) involves laking blood in distilled water, precipitating with partially neutralized trichloroacetic acid, and determining the growth promoting activity of the filtrate on *Hemophilus parainfluenzae*.

To answer the objection, three extracts were prepared and assayed in duplicate: *a*, of blood; *b*, of coenzyme solution, and *c*, in the preparation of which blood was laked in distilled water containing coenzyme equal to *b*. The assay of *c* divided by *a* plus *b*, and times 100, is taken as *per cent recovery*. The experiment was performed with five different bloods, using three different batches of media to grow the bacteria. Sufficient coenzyme was added to increase the blood assay 75 to 225 per cent. The per cent recoveries on five different days were 93, 97, 96 and 113, 101, 91.

Since the coenzymes are confined to the corpuscles, analyses should be

expressed per unit of corpuscles, and not of whole blood as has been done by Vilter, Vilter and Spies (J. A. M. A. **112**: 420, 1939) and by Querido, Lwoff and associates.

Our method measures both coenzymes. Assuming both to have equal growth-promoting activity, it was found that normal human corpuscles contain 50 to 90 gamma per ml., which is equivalent to 10 to 18 gamma of nicotinic acid. Recalculating the data of others, assuming a normal hematocrit of 45 per cent, Axelrod and Elvehjem (J. Biol. Chem. **131**: 77, 1939) found 45 to 78 gamma of cozymase per ml. of corpuscles, and Querido, Lwoff and Lataste (Compt. Rend. Soc. Biol. **130**: 1580, 1939) found 15.5 to 20 gamma of nicotinic acid.

Acute toxic effects of sodium sulfapyridine. Treatment—experimental observations. R. KOHN-RICHARDS, Abbott Laboratories, North Chicago, Ill.

Some data on the acute intravenous toxicity of sodium sulfapyridine (S.P.) were reported by Marshall and Long (J. A. M. A. **112**: 1671, 1939).

According to our results, 500 mgm/kgm. intravenously produced convulsions in dogs and were occasionally fatal; 500 mgm/kgm. i.v. in rabbits killed 25 per cent of them, 800 mgm/kgm. approximately 70 per cent, 1000 mgm/kgm. were always fatal. Deaths resulted from respiratory paralysis and pulmonary edema after convulsions. The toxic action somewhat resembled that of picrotoxin.

Therefore, an attempt was made to treat these toxic manifestations by hypnotics. Urethane was injected in various doses together with 1000 mgm/kgm. S.P. A dose of 800 mgm/kgm. urethane, which is just hypnotic if given alone, inhibited the S.P. convulsions but killed most animals by respiratory paralysis. Smaller doses of urethane were increasingly useful in saving the animals by mitigating or suppressing convulsions. The dose of 150 mgm/kgm. urethane, being without any effect alone, exerted the optimal protection in combination with S.P. If the urethane dose is lowered further the life-saving action decreases.

While 1000 mgm/kgm. S.P. is the maximum dose which can be antidoted by a single urethane injection, Pentobarbital sodium (Nembutal) in correct doses can counteract up to 1200 mgm/kgm. S.P., i.e., almost twice the M.L.D. 50. Thirty milligrams per kilogram of Nembutal, given with 1200 mgm/kgm. of S.P. produced acute respiratory paralysis though normal animals tolerate this Nembutal dose alone. Ten milligrams per kilogram of Nembutal, an ordinary non-hypnotic dose, showed maximum protective effect against S.P. Smaller amounts were less efficient. With either urethane or Nembutal in combination with S.P. it is possible to detect and differentiate two actions of the S.P., one convulsive, the other depressive. Either hypnotic is able to antagonize the first action within a certain dosage level without fatally potentiating the second. Bromides given orally in large doses a half hour before the injection of the S.P. delayed the onset of convulsions but failed to prevent death. Subcutaneous injections of morphine have so far showed no protection.

Blood and blood pressure changes following carbon arc irradiation. II.

The correlation between blood histamine and blood pressure. HENRY VON KOLNITZ (introduced by Henry Laurens). Laboratory of Physiology, Tulane University School of Medicine, New Orleans, La.

Hypertensive dogs (Goldblatt) are irradiated with a Carbon Arc, the unit dose being 35 gm.cal./sq.cm. Blood samples from the systemic circulation (saphenous) show marked increase in H-like activity following irradiation, with a simultaneous fall in arterial pressure (Am. J. Physiol. **126**: 557, 1939). Repeated irradiations maintain a lower general pressure level for several days but the histamine of systemic blood loses its correlation with blood pressure after 24 hours. Samples of blood from the irradiated skin areas are of the same order as those from the systemic circulation.

The histamine content of blood from the jugular vein, mixed with lymph, seems to correlate more closely with blood pressure changes than does that of the systemic blood. For example, 24 hours after irradiation, with the pressure maintained at 130/90 mm. Hg. from a previous level of 160/120 mm. Hg. and with 0.05 γ /cc. of H-like substance in both systemic and jugular blood, the systemic blood histamine was only 0.10 γ /cc. while the jugular was 0.5 γ /cc. Twenty-four hours later the pressure was 146/104 mm. Hg with systemic histamine 0.06 γ /cc. and jugular blood 0.10 γ /cc.

The work is being aided by a grant from the Committee on Scientific Research of the American Medical Association.

Effects of anesthetics on the blood supply to the hypothalamus. ARTHUR LAIDLAW (by invitation) and MARGARET A. KENNARD. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn. (Read by title.)¹

There is a marked difference between the appearance of fine vessels of the hypothalamus of animals under ether narcosis and those anesthetized with a barbiturate (dial, nembutal or sodium amytal). Using the method described by Finley, India ink was injected into the circulatory system of normal monkeys (*Macaca mulatta*) anesthetized by ether or by a barbituric acid anesthetic. Frontal sections of the entire brain with injected blood vessels were made and stained by Nissl's technique. The cerebral cortices of the ether animals showed slightly more pronounced blood vessels than those of animals injected while anesthetized with a barbiturate. This is in agreement with the common clinical observation that the vessels of the human cortex are dilated under ether anesthesia.

In contrast, in the hypothalamus, the vessels of animals narcotized by ether were much *less* prominent than those of animals under barbiturate anesthesia. This difference in size and number of small vessels was noticeable throughout the entire structure of the hypothalamus but was extreme in the hypothalamic nuclei (supraoptic and paraventricular). There is thus, under the influence of a barbiturate a marked specific relative dilatation of the hypothalamic vessels, particularly about the nuclei. It is known that these nuclei are richer in blood supply than any other areas of the central nervous system (Finley, Res. Publ. Ass. nerv. ment. Dis. **18**: 94, 1938). Specific dilatation of the system may be necessary during the production of sleep or of other parasympathetic phenomena integrated through the hypothalamus.

Color and super high speed cinematography of the isolated heart-lung. C. LANDIS, W. A. HUNT (by invitation) G. K. MOE (by invitation) and

¹ This investigation was aided by a grant from the Markle Foundation.

M. B. VISSCHER. Psychiatric Institute, Columbia University, New York City; Wheaton College, Norton, Mass.; and University of Minnesota, Minneapolis. (Motion picture demonstration.)

A. Color photography of the heart-lung preparation.

The several steps in the preparation of the dog heart-lung are shown in color film.

B. A temporal analysis of the events in cardiac contraction and the effects of alterations in mechanical conditions upon the action of the heart.

Motion picture photography at rates of exposure from 30 to 3000 per second have been used to demonstrate visually the mechanical changes in the completely isolated beating heart. Super high speed cinematography lends itself to an analysis of the rates of propagation of excitation between atrium and ventricle and over the ventricle itself, as well as to an analysis of the mechanical character of the contraction of the ventricle. The corkscrew motion of the ventricle in contraction can be analyzed and the dynamic effects of cardiac drugs can be demonstrated visually. The frequency and magnitude of fluid waves in the right ventricle during filling reflected from the closed pulmonary semilunar valves are demonstrated and can be analyzed. The effects of alterations in right ventricular pressure produced by changes in the pulmonary resistance are shown. These changes produce alterations in both right and left ventricular volumes. The method here demonstrated is the only one which has till now been found capable of providing a means for such analysis.

The effect of epinephrine on blood pH. P. S. LARSON. Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

Epinephrine infused intravenously causes marked alterations in blood pH. The initial effect is either a prompt fall of from 0.1-0.2 pH or a rise of approximately 0.05 pH followed by the fall to below control levels. After this period of changing pH (occupying 10-20 minutes) the pH remains fairly constant at its new low level for the duration of the epinephrine administration (10-20 minutes in these experiments). On cessation of infusion the blood pH gradually rises and approaches the pre-infusion level in 30 minutes.

The accompanying table includes an experiment illustrative of each type of response. Cats under dial anesthesia were used; each cat received 0.005 mgm. of epinephrine per kilogram of body weight per minute.

CAT NUM- BER	CONTROLS, 10 MIN. INTERVAL	pH DURING EPINEPHRINE INFUSION					pH CHANGES AFTER INJECTION		
		2 Min.	7 Min.	12 Min.	20 Min.	30 Min.	10 Min.	20 Min.	30 Min.
1	7.41-7.40	7.35	7.29	7.23	7.22	7.22	7.33	7.37	7.39
2	7.42-7.43	7.48	7.47	7.32	7.30	7.29	7.32	7.36	7.41

The above effects are seemingly independent of changes in pulmonary ventilation, for they occur during constant artificial respiration. The possible relation of the blood pH changes in response to epinephrine to the simultaneously occurring plasma potassium variations will be discussed.

The effect of intra-intestinal pressure on blood flow through the intestine.

HAMPDEN LAWSON and JACK CHUMLEY (by invitation). Department

of Physiology, University of Louisville School of Medicine, Louisville, Ky.

Arterial flow into short (6-12 cm.) loops of ileum was studied in barbitalized dogs by a modification of the differential manometer method of Lawson and Holt (J. Lab. and Clin. Med. **24**: 639, 1939). Venous outflow was also recorded in some cases by cannulation of the mesenteric vein draining the loop (chlorazol fast pink as anticoagulant) and signaling drops.

On inflation of a large balloon within the loop, arterial inflow was reduced, and venous outflow increased, for a period of 10 to 30 sec., the change in both flows being roughly proportional to the distending pressure within the balloon up to the level of mean carotid pressure. If balloon pressure was less than 30 mm. Hg, both flows usually then returned to normal, and remained without further change until deflation. If, however, balloon pressure was above 30 mm. Hg, both flows returned to a value below normal, the reduction in flow being roughly proportional to the distention pressure up to the level of mean carotid pressure. On deflation there was a brief period of increased inflow and decreased outflow, apparently a reversal of the changes seen immediately following inflation. The immediate effects of inflation and deflation are probably due to capacity changes in the vascular system, since inflow and outflow are oppositely affected. Following deflation after distentions which had caused persistent reduction in flow, the capacity effect on flow was followed by a period in which both inflow and outflow were increased. The latter is probably a type of reactive hyperemia, since in degree and duration the increased flow seems to be related to the amount of reduction in flow during the distention.

These data probably have no bearing on any clinical condition in which distention may reduce blood flow through the intestine, since the observations were made with the belly opened so that no rise in intra-abdominal pressure occurred, and since the distentions studied were not maintained for longer than 10 minutes.

The effect of calcium and phosphorus on the retention of lead by the growing organism. LUDWIG G. LEDERER and FRANKLIN C. BING (introduced by C. A. Dragstedt). Department of Physiology and Pharmacology, Northwestern University School of Medicine, Chicago, Ill.

Young albino rats were fed on diets containing different levels and ratios of calcium and phosphorus and constant amounts of lead. In one series the level of phosphorus was maintained constant at 0.5 per cent, and the amount of calcium was made 0.12, 0.25, 0.50, 1.00 and 2.00 per cent. In another series the ratio of Ca:P was kept constant at 2.0 and the amount of calcium in the diet was made 0.25, 1.00, and 2.00 per cent. The lead content of each diet was 0.01 per cent. The rats were maintained on these diets for three weeks, when roentgenograms of the bodies were taken. The roentgenograms revealed no evidence of rickets in any of the animals, although in the groups receiving low amounts of calcium the bones appeared to be poorly nourished. The lead content of the femurs varied considerably in the different groups, depending on the calcium content of the diet. The bones of the animals receiving higher amounts of calcium contained lower amounts of lead. The lead retained by the kidneys

likewise varied inversely with the calcium content of the diet. There was no effect that could be attributed to the phosphorus content of the diet or to the ratio of the calcium to phosphorus.

When rats were maintained on diets in which the level of calcium was either 0.25 or 2.00 per cent and a solution containing 0.1 mg. of lead was injected intraperitoneally daily, the lead contents of the femurs, kidneys, and livers were high but were not essentially different in either group. It thus appears that calcium interferes with the absorption of lead from the intestinal tract, probably by the formation of a complex insoluble salt. These observations are of interest in connection with the selection of diets which will tend to prevent an accumulation in the growing body of the minute amounts of lead which are present in all foods.

Some effects of bulbocapnine on the peripheral vascular system. CHESTER E. LEESE, ALMA FOGELBERG (by invitation) and HOWARD MAMLET (by invitation). Department of Physiology, The George Washington University School of Medicine, Washington, D. C.

Previous studies by the authors have suggested vascular effects produced by bulbocapnine which precede and accompany the catatonic picture ascribed by most writers to the influence of the drug upon the central nervous system. Twenty rats were prepared for histological study of circulatory effects. Experimental animals received 80 mg. per kg. of bulbocapnine in solution intraperitoneally and the controls received a like volume of Ringer's solution. Following the onset of catalepsy the experimental animal and its control were injected with filtered Higgin's India Ink into the heart, the amount given being equal to 10 per cent of the calculated blood volume. After five minutes, the entire animal was fixed in trichloroacetic acid. Dioxan and paraffin techniques were employed.

The following observations have been made:

1. The zona reticularis of the adrenal cortex showed the greatest change of all tissues studied thus far. Sinusoids become so large that they appear to completely merge.

2. The liver and adrenal medulla showed extensive congestion.

3. Pancreatic islets showed congestion, while the rest of the pancreas was little affected.

4. Capillaries of the gastro intestinal mucosa were dilated. Crypt and villi vessels were frequently engorged.

5. Venous sinuses of the spleen were congested and the vessels supplying the Malpighian body were dilated.

6. Kidney glomeruli were often congested and the nature of ink distribution in the kidneys of experimental animals differed from that of the controls.

7. Coronary vessels appeared congested in some animals and unchanged in others. The vasa vasorum showed general dilation.

8. Both thyroid and parathyroid glands showed the vaso-dilating influence of bulbocapnine.

9. The lung, tongue, striated muscle, and skin appeared to be least affected.

10. It is concluded that all tissues thus far studied show some increase in the number of vessels filled and an increased vessel dilation as judged by the amount and distribution of ink. The greatest vaso-dilation and congestion appeared in the adrenal cortex, liver, and adrenal medulla.

The influence of added dextrose and of insulin on hepatic glycogenolysis in vitro. R. LEVINE (by invitation), M. TAUBENHAUS (by invitation) and SAMUEL SOSKIN. Department of Metabolism and Endocrinology, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Chicago, Ill.

The homeostatic mechanism for the regulation of the blood sugar level, whereby the liver diminishes its output of sugar to the blood in response to hyperglycemia, was first postulated from studies of the dextrose tolerance curve. This phenomenon was later demonstrated by direct observation of the intake and output of sugar by the liver in intact animals. In preliminary reports, we have recently shown that added dextrose and insulin decrease the rate of appearance of free sugar in glycogenolyzing liver brei. This indicated that hepatic homeostasis depended upon the effects of glucose and insulin upon the enzyme systems involved in glycogenolysis. The present report advances further evidence regarding the mechanism of these inhibitory actions.

The use of various preparations of minced liver and the employment of specific inhibitors of amylase activity (Ascorbic Acid) and of phosphorylase (Phlorhidzin), make it possible to conclude that amylase plays no significant rôle in hepatic glycogenolysis under physiologic conditions. The study of the effects of dextrose and of insulin on glycogen breakdown by phosphorylation, indicate that glycogen phosphorylase is inhibited by dextrose. The latter displaces glycogen in the competition for the phosphorylating systems. Insulin influences the phosphorylating mechanisms to re-inforce this effect.

*Control of electrical and oxidative activity of brain by temperature.*¹ B. LIBET (by invitation), J. F. FAZEKAS (by invitation), A. M. MEIROWSKY (by invitation), E. H. CAMPBELL (by invitation) and H. E. HIMWICH. Departments of Physiology and Pharmacology and of Surgery, Albany Medical College, Union University, Albany, N. Y. (Read by title.)

The effects of lowered temperature on electrical activity and arterio-venous oxygen differences of the brain were studied in dogs and cats. Effect of an anesthetic was eliminated by placing electrodes during ether anesthesia and installing curare paralysis and artificial respiration when ether was blown off. Checks for possible anoxemia, altered blood carbon dioxide content, blood pressure, and cerebral blood flow were made.

Electrical activity of cerebral cortex, posterior hypothalamus, and medulla decreased in frequency and amplitude with cold, but some activity persisted in all portions of the brain until 15°C., at which temperature other parts of the body (cord, heart, etc.) apparently cease function. This is in marked contrast to the depression caused by anoxia or hypoglycemia when cerebral electrical activity stops as the rest of the body still maintains function.

Synchrony (regularity of waves) generally improved as the temperature dropped several degrees, but with further fall of temperature synchrony progressively decreased. Q_{10} , when measurable, is from 2 to 2.5 (similar to that found for oxygen consumption of cortical tissues *in vitro*). The drop in frequency is not regular and sudden changes in rhythms occur. At 23° to 20°C., trains of large convulsive type waves commonly develop, and these gradually decrease to zero at 15°C. These waves do not appear

¹ Aided by a grant from the Child Neurology Research (Friedsam Foundation).

to be due to anoxia. It is significant that electrical activity is still considerable at 20°C., when the cerebral arterio-venous oxygen difference, even with a reduced cerebral blood flow is only $1\frac{1}{2}$ to 2 volumes per cent. In other experiments in which pentobarbital anesthesia was used, electrical activity not only is slower at body temperature, but ceases at 25°C. when the animal usually succumbs. Apparently pentobarbital acted synergistically with cold.

Constant brain potentials and non-neural transmission. B. LIBET (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Chicago, Ill.

The isolated frog brain, after soaking in 0.5 per cent caffeine, exhibits large (to 1 mv.), mainly surface positive, slowly spreading (5 cm. a sec.) potential waves. These "caffeine waves" still travel when synaptic transmission is blocked by nicotine and can even cross a complete anatomical transection of the forebrain; so that spread involves some mechanism in addition to the usual impulse-synapse transmission. We have postulated the flow of intercellular currents as follows.

A steady potential gradient along the neurone soma, axonal end usually positive to dendritic, is discharged to give the surface-positive "caffeine wave." Adjacent cells can then "depolarize" by intercellular currents and the wave spreads. The potential across a sheet of cerebral cells was measured and found to be several millivolts in size and directed as predicted. This constant potential along the cell body, the "somatic potential," is separate from the familiar one across the membrane. Alterations in size and direction of the somatic potential, whether spontaneous or induced by polarization, produce the changes in size, duration, velocity, and "after-potentials" of the caffeine waves which the theory predicts.

In the uncaffeinated brain, also, "depolarizing" the somatic potential increases activity and may initiate strong waves in a previously quiescent preparation. Polarizing through the length of the brain shifts the locus of origin of caffeine waves towards the region of reduced somatic potential. An additional factor of excitability, aside from the magnitude of the resting somatic potential, must, however, also be present. Several similarities to nerve conduction are striking.

It is concluded: one neurone can affect another's activity, aside from nerve impulses, through intercellular electric currents; the somatic potential and its alterations are important in cell activity and interaction; and potential gradients over a sheet of neurones can determine where activity originates and may, therefore, aid in controlling the shifting patterns of brain activity.

The response of the newborn to respiratory stimulants. K. T. LIM (by invitation) and FRANKLIN F. SYNDER. Departments of Obstetrics and Gynecology and of Pharmacology, University of Chicago, Chicago, Ill.

The present experiments represent an attempt to determine the changes in respiratory activity of the newborn following administration of respiratory stimulants, including caffeine, coramine, alpha-lobelin, metrazol, and cyanide. Numerous reports regarding the rôle of respiratory stimulants at the time of birth, especially in the human, have been difficult to evaluate since parturition is complicated frequently by various factors

which may alter respiratory activity such as anoxemia, anesthesia and trauma.

The present observations are based upon newborn rabbits studied immediately following delivery by hysterotomy at 31 days of gestation, or within a few hours after birth at term, i.e., 32 days. Forty-four animals obtained from 18 litters were observed. Respiratory tracings were made by means of a small plethysmograph connected with a recording tambour. Pentobarbital sodium, 30 milligrams per kilogram body weight, was injected subcutaneously to facilitate the recording of respiratory movements. Respiratory stimulants were injected intravenously.

The minimal effective dose and the minimal lethal or convulsive dose were determined for each substance. Results showed that response by increase in rate or depth of breathing was brief, lasting less than a minute. There was a narrow margin between the dosage level at which stimulation appeared and the dosage at which convulsions or death occurred. Thus, with lobelin convulsions followed a dose which was twice the minimal effective amount; and with caffeine, metrazol, coramine, and cyanide convulsions followed a dose of three times the minimal effective amount. Furthermore, death frequently followed the onset of convulsions resulting from the administration of alpha-lobelin, caffeine or coramine.

The effect of parathyroid-extract injection upon hearing. ELI A. LIPMAN (by invitation), FRED A. METTLER and ELMER CULLER. Department of Psychology, University of Rochester and Department of Anatomy, University of Georgia School of Medicine. (Read by title.)

Injection of parathyroid extract into young dogs causes an increase of calcium metabolism, resulting in a condition resembling osteitis fibrosa cystica, with resorption of bone and formation of osteoid tissue (Jaffe and Bodansky, J. Exper. Med. 52: 669, 1930). The present experiment was designed to determine the effect of excessive parathyroid injection upon the animal's hearing.

After young dogs have been trained by the conditioned-response technique, their normal hearing thresholds are determined. The animals are then placed on a low calcium diet and Paroidin (Parke, Davis & Company) is injected, beginning with small doses (two units daily) which are then gradually increased to 100 or 125 units each day. Since tolerance varies greatly from animal to animal, and since dosage must also be adjusted to the period of injection, great care is required. If the tolerance-level is properly observed, animals can be kept for long periods of time, whereas excessive dosages are soon fatal.

Some animals have now been receiving injections for a period of two years. At first no losses appeared, but after high dosages were reached, marked impairment of hearing was found. These figures are representative:

NUMBER	125 CYCLES	1000 CYCLES	8000 CYCLES
	db.	db.	db.
19	20	20	30
23	19	12	7
25	25	30	21

The magnitude of these losses clearly indicates that one effect of excessive parathyroid is impairment of hearing; but until the histological examination is completed, we cannot say what bony changes have occurred.

The excitation of motoneurons by descending fibers of the ventral columns.

DAVID P. C. LLOYD. Department of the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Single shock excitation of the ventral columns of the thoracic cord of the cat results in the appearance of two alpha spikes in the lumbar cord. The first is directly conducted; the second, later by less than a millisecond, represents secondary descending neurons as shown by facilitation, inhibition and susceptibility to asphyxia. In the ventral horn the descending spikes are followed by a period of internuncial activity.

The discharge of motoneurons following the cord stimulus occurs only after the passage of one or more local relays in addition to the primary and secondary descending neurons. These latter, however, do have a direct subliminal excitatory effect upon the motoneurons. The motoneuron discharge itself may last throughout the period of local internuncial activity.

Repetition of the cord stimulus produces sharply synchronized motoneuron discharges whenever a volley in the secondary descending neurons reaches the motoneurons during the local internuncial activity. In this way three synchronized motoneuron discharges can be produced by a train of four cord stimuli. The number of such discharges which can be obtained with longer tetani is limited by the summation of inhibitory effects that abolishes the necessary background of local internuncial activity. The discharge in the controlling secondary descending neurons also is reduced by inhibition higher in the cord.

Synchronization of the fringe of subliminally excited motoneurons occurs together with the synchronization of motoneuron discharge. The duration of the synchronized subliminal fringe is so short that excitation by a dorsal root volley arriving at the motoneurons through successively longer reflex arcs are to a considerable extent differentially facilitated. Perfect differentiation is prevented by dispersion introduced into the multineuronal system by differences in conduction rates and synaptic delays. However, as little as 0.3 msec. alteration in the arrival of dorsal root impulses with respect to impulses of the controlling secondary descending neurons will change the whole pattern of the resulting facilitated motoneuron discharge. Since the peaks of the facilitation curves in these experiments do not outlast the synchronized motoneuron discharges, it is evident that short time course excitatory actions must be of great importance in the spinal cord.

Changes in equilibration as a basis for conditional movements. HANS LÖWENBACH (introduced by W. Horsley Gantt). Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md.

The ability to maintain equilibrium can be disturbed in vertebrates by stimulation of either the optic, the vestibular or the kinaesthetic apparatus. Whether or not it is possible to condition a reaction to an auditory stimulus if such stimulus previously had been accompanied by a change in the slope of the base on which the subject rests was the purpose of these experiments. An oak board laid crosswise over an iron bar was

kept in balance by springs. The excursions of this platform produced by the change of the subject's center of gravity were recorded. To exclude visual orientation human subjects (5) were blindfolded; dogs (4) were placed in a box. The conditioned stimulus was given for 5 sec. After 3 sec. the platform was quickly tilted through 10-20 degrees. Soon a conditioned movement towards the opposite side appeared before the tilting. This reaction was constant until the 50th trial when it gradually disappeared, and appeared again after the 170th trial remaining constant until the 500th trial. Extinction and differentiation were successfully tried. These experiments give further evidence that the maintenance of equilibrium, in the higher vertebrates, is not a solely subcortical function.

Phosphorus metabolism in the musculature of dystrophic vitamin E-deficient rats. GWEI DJEN LU (by invitation), GLADYS A. EMERSON (by invitation) and HERBERT M. EVANS. Institute of Experimental Biology, University of California, Berkeley.

Chemical studies were made of the musculature of a single hind limb of eleven adult rats rendered dystrophic from deficiency in vitamin E. As controls, similar studies were made of the musculature of seven animals of the same age maintained on a natural food diet.

Analyses were made of the following phosphorus fractions: inorganic phosphate, creatine phosphate, total acid soluble phosphate and total phosphate.

The affected musculature showed slightly less inorganic phosphorus, no change in creatine phosphate, a marked decrease in total acid soluble phosphate and in total phosphate. The ratio of creatine-phosphate-phosphorus to total acid soluble phosphorus was therefore increased.

The ability of the affected musculature to phosphorylate glycogen was decreased approximately 46 per cent when compared with the behavior of normal musculature.

The relative glycosuric effect of thyroid and anterior pituitary. F. D. W. LUKENS and F. C. DOHAN (by invitation). George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia. (Read by title.)

Two partially depancreatized cats were used as the test animals. In these experiments, the pancreatic remnants were small, the animals had slight glycosuria for some time after operation and then became sugar free.

Glycosuric action of thyroid and anterior pituitary extract (APE)

CAT	WEIGHT	TOTAL MEAT	PERIOD OF TREATMENT	RAW THYROID	WEIGHT AFTER THYROID INGESTION	APE	MAXIMUM GLYCOSURIA DURING TREATMENT
	kgm.	grams per day	days	grams per day	kgm.	cc. per day	grams per day
6	2.65	200	21	40	2.25		4.4
	2.70	200	2			5	9.6
	2.35	140	3			5	10.3
29	2.75	140	3	40	2.35	2	8.5
	2.60	200	21				0
	2.95	100	3			5	11.1

This suggested that they were quite susceptible to any metabolic stress. Thyroid was given as the raw gland, minced with their beef feedings. The adequacy of the dosage was indicated by the weight loss which occurred on a large diet (200 grams total meat). Tachycardia, increased hunger and nervousness were also noted. Anterior pituitary extract (APE) was injected intraperitoneally as the fresh saline suspension. The results of these two agents are compared in the table.

Cat 6 frequently had glycosuria of 2 to 4 grams per day without any treatment. Although in the experiments cited it was sugar free at the beginning of each treatment, the excretion of 4.4 grams is of doubtful significance. Cat 29 was sugar free except during the injection of APE.

In addition, these two cats, and a third animal, were given courses of thyroid extract as pills by mouth. Doses of 5 to 10 grains per day for 3 weeks produced no weight loss and no effect on the urine glucose.

Absorption of chloral hydrate through skin. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

Aqueous and hydroalcoholic solutions of chloral were applied to the unshaved skin of mice. Care was taken to prevent licking and consequent absorption through mouth. Aqueous solutions produced no sign of absorption. Application of a 5 per cent solution of chloral in 90 per cent ethyl alcohol was followed first by depression and then by hypnosis, indicating absorption through skin. Absorption was facilitated when 1.5 per cent chloral in 30 per cent alcohol was employed. Application of such solution was followed by definite narcosis from which animals recovered in several hours. Various concentrations of alcohol alone produced no depression.

Clinical blood pharmacology after exposure to x-rays. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

In a previous communication (Am. J. Roentgenol. **41**: 709, 1939) the author described the characteristic phytotoxic reaction of blood taken from different species of normal animals after their exposure to x-rays. The sera of such blood specimens were much more toxic for living seedlings than the normal blood sera obtained from the same animals before initiation of roentgen therapy. In the present study, the writer examined blood specimens of 20 human patients at a well-known hospital before and after their treatment with x-rays for various diseases. The subjects thus studied so far include cases of malignant disease as well as those suffering from benign tumors and inflammatory conditions. Phytopharmacological examination of the blood sera obtained from these patients one to three days after treatment with x-rays revealed that they had a marked toxicity as compared with sera drawn from the same persons before such therapy was instituted. Specimens of blood obtained from several technicians, who were in apparently good health and had been working in the x-ray department for considerable periods of time, also exhibited a definitely phytotoxic reaction.

Observations on reproduction after repeated injections of cobra venom. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

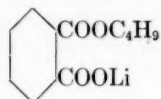
Numerous male and female mice which had received sublethal doses of cobra venom solution (H. W. & D.) in connection with the biological assay of this drug were allowed to mate. In all cases the females passed through a normal period of gestation and gave birth to normal litters, their offspring on reaching maturity not being distinguishable from that of controls. Six adult female and two male guinea pigs which had been repeatedly injected with 5 mouse unit doses of cobra venom were allowed to mate. These female animals also gave birth to normal litters. A series of female rabbits, on which was studied the effect of injections of 5 mouse units of cobra venom (ordinary therapeutic dose for humans) five times a week for periods varying from 12 to 24 weeks, also passed through normal pregnancies and gave birth to normal litters. Three of the rabbits underwent more than one pregnancy with the same favorable results. These observations indicate that cobra venom does not interfere with reproduction or normal course of pregnancy in rabbits, guinea pigs and mice, and the findings agree with reports received concerning human patients subjected to cobra venom therapy during pregnancy.

Pathological findings after repeated injections of cobra venom. DAVID I. MACHT and DOROTHY J. BROOKS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

Rats, and especially rabbits, were repeatedly injected with from 5 to 10 mouse units of cobra venom solution (H. W. & D.). Some of the animals were sacrificed and sections were made of different organs for histological examination. From 300 to 800 mouse units had been injected into some of the rabbits before microscopic examination of the organs was made. Sections of the heart, liver, spleen, kidney and brain thus obtained and stained with Harris hemotoxylin stain showed under the microscope no appreciable pathological changes when compared with controls. A more detailed and extensive study of brain and spinal cord treated with special differential stains for neuropathological examination is in progress. Physiological-pathological studies on living rabbits treated in the manner described above revealed that neither their kidney nor liver function was impaired, and that there were no characteristic blood changes.

Pharmacology of lithium-butyl-phthalate. DAVID I. MACHT and H. A. B. DUNNING, JR. (by invitation). Pharmacological and Chemical Research Laboratories, Hynson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

This compound, soluble both in oil and water, was prepared for the use of a physical chemist in studies on bombardment of atoms. A pharmacological examination of the compound was deemed of interest and was made on mice, rats, rabbits, cats and on living seedlings of *Lupinus albus*.



The toxicity of lithium-butyl-phthalate was found to be very low. Intraperitoneal or subcutaneous injections of one cc. of a 10 per cent solution

in oil or saline produced no effect on mice. Injections of 5 to 10 cc. of a one per cent solution were not poisonous for rats (250 grams). One hundred milligrams of lithium-butyl-phthalate in saline solution, injected intravenously in rabbits, produced no impairment of kidney function or other toxic effect. Repeated injections (totaling 250 mg.) into femoral vein of cats under ether had no effect on either respiration or circulation. In contrast to the results derived from the zoöpharmacological experiments, an interesting finding was made on phytopharmacological examination of the compound. Lithium-butyl-phthalate is very toxic for root growth of *Lupinus albus* seedlings as compared with controls grown in potassium- and sodium-butyl phthalates, respectively. When a comparison was made of the inorganic salts—namely, the sulphates of lithium, potassium and sodium, respectively—it was found that this toxicity was inherent, in part at least, in the lithium atom.

Biochemical and pharmacological differentiation of cobra venom from other snake venoms. DAVID I. MACHT and WILTON G. HARDEN (by invitation). Chemical and Pharmacological Research Laboratories, Hyson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

The composition of snake venoms differs in respect to their proportion of ingredients common to most. The principal constituents of snake venoms are hemorrhagins, hemolysins, coagulants and anticoagulants, proteolytic substances and neurotoxins. Cobra venom consists largely of neurotoxin, which is probably not a protein and can be separated from other constituents by suitable filters. A solution of cobra venom neurotoxin, made from the venoms of *Naia tripudians*, *Naia naia* and *Naia haji*, according to Van Esvald's method (Biochem. Ztschr. **283**: 343, 1936), was compared with solutions similarly prepared from equal weights of dried venom of Russell's viper, *Bungarus fasciatus*, *Agkistrodon* and various *Crotalidae*, respectively. One hundred milligrams of dry scales, yielding a final solution of 450 cc. of neurotoxin, when assayed on mice, revealed a potency of 10 to 12 mouse units per cubic centimeter in case of venoms from the three species of cobra but less than one mouse unit to the cubic centimeter in case of the respective venoms from the other species of snakes. When examined phytopharmacologically, one per cent cobra neurotoxin solutions, prepared from above-mentioned solutions, gave a phytotoxic reading of 50 to 60 per cent, while similar one per cent solutions of respective venoms of the other snakes exhibited no toxicity when tested in seedlings of *Lupinus albus* by the senior author's well-known methods. When tested according to Noc's method (Ann. Inst. Pasteur **19**: 209, 1905), the venoms of the other snakes were found to have much more proteolytic activity than cobra venom. Cobra venom neurotoxin was heated for 15 to 30 minutes at 100°C without much impairment of its activity. The potency of the other snake venoms is destroyed by heating them to but 70°C. Unfiltered solutions made from aliquot parts by weight of the three species of cobra venom proved much more poisonous for mice than venoms of *Bungarus*, Russell's viper, *Bothrops*, *Agkistrodon* and the *Crotalidae*.

Comparison of effect of cobra venom and opiates on olfactory sense. DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological

Research Laboratory, Hynson, Westcott and Dunning, Inc., and Psychological Laboratory, Johns Hopkins University, Baltimore, Md. (Read by title.)

Quantitative comparative studies on olfaction were made by employing Zwaardemaker's classical method of testing olfactory responses (C. W. Nagel, *Physiologie des Menschen*. Handbuch der Physiologie des Menschen, 3: 603 ff., 1904). Human subjects were injected with cobra venom (5 mouse units), morphine (1/10 to 1/8 gr.), codeine (1/4 gr.), and dilaudid (1/64 gr.), respectively. By means of the olfactometer, with which red rubber and Russian leather standards were used, it was found that the opiates generally depressed the sense of smell while cobra venom had no such effect, or as often happened, rendered it more acute. These observations support the findings obtained in the authors' previous psychological experiments on vision and audition, which indicated that the analgesic action of cobra venom is localized below the cortex and probably in the hypothalamus.

Effect of cobra venom and the opium alkaloids on the psychogalvanic reflex.

DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., and Psychological Laboratory, Johns Hopkins University, Baltimore, Md. (Read by title.)

The writers studied the psychogalvanic reflexes with electrodes applied to the palm of the hand, and the effect thereon of injections of cobra venom and various opiates. The delicately standardized apparatus employed was the affectometer devised by Ruckmick and rendered available through the courtesy of the Maico Company. Various drugs were given to normal human subjects by intramuscular injection and others were administered by mouth. The authors have observed a difference between effects of ordinary doses of cobra venom and of morphine, codeine and dilaudid, respectively. Injections of morphine (1/10 to 1/8 gr.), codeine (1/4 gr.) and dilaudid (1/64 gr.) are succeeded by a marked rise in the affectometer reading in ohms, the increase being as great as 30,000 ohms. On the other hand, cobra venom effects quite as marked a drop in readings made on the subjects tested up to the present time. These findings are being analyzed in an endeavor to localize more accurately the seat of action of the drugs on the nervous system. So far they confirm the view already expressed by the authors that the opiates depress the cortex while the action of cobra venom is exerted on synapses lower down.

Clinical blood morphology and chemistry after repeated doses of cobra venom.

DAVID I. MACHT, SOLOMON SHERMAN (by invitation) and DOROTHY J. BROOKS (by invitation). Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Md. (Read by title.)

A complete and differential blood count of erythrocytes and leukocytes, hemoglobin index and chemical determination of total urea, urea nitrogen and blood sugar were made by standard methods on ten rabbits, three of which were normal controls. The other seven rabbits were repeatedly injected with from 5 to 10 mouse units of cobra venom solution (H. W. & D.) at the rate of five times a week for periods ranging from three to eighteen weeks. As compared with the controls, the animals treated with cobra venom revealed no injurious effect in blood picture and blood chem-

istry, no specific changes being noted in the absolute blood count, differential blood count, hemoglobin content, blood sugar and urea nitrogen. No hemolytic changes could be detected nor was the coagulation time affected. In both controls and treated animals there were individual variations which could not in any way be correlated with injections of the drug.

The effect of diet upon renal hypertension. I. MACLACHLAN (by invitation) and N. B. TAYLOR. Department of Physiology, University of Toronto, Toronto, Canada. (Read by title.)

It was reported in a recent paper (Greenwood, Massim and Taylor, Can. M. A. J. 41: 443, 1939) that if one kidney is enclosed in a cast and the other removed, pronounced arterial hypertension appears in from 24 to 48 hours and persists indefinitely. The cast prevents, presumably, the hypertrophy of one kidney which occurs normally as a compensatory process after excision of its fellow. This result supported our belief that the renal pressor substance is liberated as a response to a disproportionality between the work which the kidney is called upon to perform and its blood flow. In other words, hypertension produced in this way or by constriction of the renal artery is, it is suggested, part of a physiological mechanism whereby the kidney controls its blood flow in accordance with functional demands. Therefore, an animal with a moderate degree of hypertension should show a further rise upon increasing its renal work. When the diet of an animal with a cast upon its only kidney (B.P. 150 mm. Hg) was changed from dog biscuits to one composed of 800 gm. of meat daily a pronounced rise in blood pressure (from 151 mm.-176 mm. Hg) occurred within 24 hours accompanied by serious hypertensive effects, viz., bilateral retinal detachment, and cerebral symptoms. Upon the animal's return to the low protein diet the pressure subsided gradually though it did not reach its previous level by the end of a week. The diet was changed from biscuits to meat and back again to biscuits several times, each dietary period lasting for 4 days. In each instance the blood pressure was from 30 mm. to 60 mm. higher upon the meat than upon the biscuit diet. The rise in pressure occurred too rapidly to be accounted for by an increase in body weight as suggested in a recent paper by Wood and Cash (Wood and Cash, Am. Int. Med. 13: 81, 1939). Indeed, the body weight showed a steady decline throughout the course of the experiments. Increasing the work of the kidney by feeding urea (50 grams daily) was followed by a hypertensive effect of the same magnitude as that caused by the high protein diet.

*The metabolism of human spermatozoa.*¹ JOHN MACLEOD (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York City. (Read by title.)

The metabolism of human spermatozoa in *Ringer-glucose* is almost exclusively glycolytic. Any O₂ consumption is sporadic, of low magnitude, and is not in any sense quantitative. There is no spectroscopic evidence of cytochrome in this tissue. Sperm motility at 38°C. is impaired markedly in the presence of O₂ or air but is unaffected in N₂. This effect in the presence of O₂ is gradual and may only become apparent from four to six hours after ejaculation of the cells. The immobilizing effect of air or of

¹ Supported by a grant from the National Committee on Maternal Health.

O₂ is apparent at room temperature but is more definite and appears more rapidly at 38°C. It is possible that these cells produce H₂O₂ and there is definite evidence that a trace of hemoglobin added to the cells will protect motility in the presence of O₂. Catalase has not yet been used. The addition of methylene blue or of pyocyanin does not increase or stabilize the O₂ consumption in Ringer-glucose. On the contrary, both dyes have a decidedly toxic effect upon the cells in the presence of O₂, an effect which is almost completely absent in N₂ when equivalent concentrations of dye are used.

In seminal fluid, the cells produce approximately twice the amount of lactic acid (aerobically and anaerobically) as do the same cells in Ringer-glucose. Since, in the cases studied, motility in both media is about the same, the increased lactic acid production may be related to the relatively high viscosity of seminal fluid and not to any specific stimulating substance in the seminal fluid itself.

Further studies on cortical potentials during hypoglycemia produced by hepatectomy with and without abdominal visceration. STEPHEN MADDOCK (introduced by F. A. Gibbs). Boston City Hospital, Boston, Mass.

In 1939 Maddock, Hawkins and Holmes published experiments on rabbits in which the liver, kidneys and intra-abdominal viscera were removed. These animals become hypoglycemic within 4 hours. Electrodes were placed on the cortex and the cortical potentials studied with a capacity-coupled amplifier and recorded by a Grass oscillograph. Attention was directed chiefly to various substances which were thought to be part of the "glucose cycle." It was found that none of the substances available were capable of restoring the normal cortical potentials during hypoglycemia except glucose, mannose and maltose.

The present study was undertaken in order to determine whether there were any other carbohydrates which might be utilized by the brain and also to discover whether the kidneys or other abdominal viscera play any role in the synthesis of glucose or other effective materials. The experiments were performed on rabbits. It has been found that hepatectomy alone does not alter the results obtained in the previous study. Many new compounds have been tested but none of these have proved efficacious with the exception of levulose which is effective following hepatectomy alone as had previously been described by others. It has not as yet been possible to obtain all the aldo-hexoses but of those studied only d-glucose and d-mannose are capable of supporting normal cortical rhythm. None of the poly-saccharides except maltose and glycogen were effective. Both of these substances must be given early enough to allow hydrolysis into glucose to occur.

Glycogen does not continue its effectiveness for as long a period as glucose. In the late stages following hepatectomy glucose can no longer be phosphorylated, due to the disappearance of enzymes. Apparently the enzyme which is lacking is a phosphatase, but other cofactors cannot be excluded. Blood from such animals (either dogs or rabbits) continues to split glycogen in vitro but glucose disappears very slowly or not at all. When normal blood is added to blood from hepatectomized animals the rate of disappearance of glucose returns to normal.

Response of medullary respiratory action potentials to modifications of respiration. CONWAY S. MAGEE (by invitation), JOHN W. BRICKER (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Previous reports have shown the neurohistological source of central action potentials in phase with respiration and the three basic patterns of central respiratory discharge. In dogs, decerebrated under evipal, we have studied the modifications of these respiratory discharges from various histological sources as the result of procedures modifying respiration.

When inspiratory mechanical asphyxia was applied while inspiratory potentials were being recorded, 80 per cent of the experiments showed a marked augmentation of the sensory, motor and intermediate cell discharge. The response of expiratory potentials to inspiratory mechanical asphyxia was more variable although a common effect was also an augmentation of discharge.

Expiratory mechanical asphyxia had three main effects: It always increased the expiratory potentials; it frequently increased the non-rhythmic potentials; and occasionally increased the inspiratory potentials.

In half of the experiments artificial ventilation produced no immediate noticeable change in respiratory potentials. In some experiments the non-rhythmic potentials were increased. In other experiments expiratory potentials were elicited by artificial inflation.

The responses to these and other procedures such as the administration of NaCN, Na₂CO₃, and CO₂ have been used to study the integration of the respiratory act.

Effect of training on the performance of ischemic human muscle. GEORGE L. MAISON. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

These studies seek to answer the following questions: Does training appear in the performance record of ischemic human muscle worked daily? Does training of circulated muscle improve the ability of the same muscle in ischemia?

Volunteers worked the extensor digitorum communis muscle of each arm to exhaustion once each day. The ergograph automatically signified the length of stroke to be taken (see Demonstration). Exhaustion was defined as that degree of fatigue which prevented reaching this stroke length in the given time interval. 5 strokes were made every 5 seconds. Ischemia was produced by an arm-cuff inflated instantaneously from a large reservoir at a pressure above 220 mm. Hg.

12 muscles without previous ergograph experience were worked daily in the ischemic state. There was a gradual rise in total work done each day during a preliminary period of 10 to 18 days in 6 cases. Thereafter a plateau level was reached. The other 6 cases produced a plateau from the start. Ten of these records were continued for 2½ to 4 months. In 6 cases no improvement occurred. In 4 there was a very slow rise which had reached only 108 to 120 per cent of original plateau ability when recording stopped. In all 10 cases the contralateral muscle which was worked daily in circulated condition showed marked training.

Eight untrained muscles in 5 subjects were worked ischemically once each day until it was thought a plateau had been reached. Then work was done daily for 2 months with limb circulated. Work ability of circu-

lated muscle was multiplied several fold by the training in each case. After that, daily ischemic work was resumed and plateau obtained. In 5 muscles the variation between the pre- and post-training plateaus was not more than 7 per cent of original ability (average +2 per cent). The other 3 muscles showed a gain of 20 to 30 per cent. The original plateau was not of sufficient duration to prove that the improvement in these 3 muscles was a result of circulated training.

Laboratory apparatus. GEORGE L. MAISON and A. G. BROEKER (by invitation). Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo. (Demonstration.)

The following units will be demonstrated:

1. A compact ergograph for student use which enumerates the total distance in cm. through which load is lifted and permits recording of strokes on drum. Overall size of ergograph is 14" x 6" x 4", thus it is readily stored.
2. An ergograph which enumerates strokes made and total distance load is moved. It permits control of stroke length.
3. A shielded stimulating electrode for cut nerves. The design prevents short circuiting of the poles and preserves the moisture of the nerve.

Dark adaptation under controlled dietary vitamin A conditions. JOSEPH MANDELBAUM (by invitation) and SELIG HECHT. Laboratory of Biophysics, Columbia University, New York City.

The dark adaptation of 17 young men during controlled vitamin A diets was studied with the Hecht-Shlaer adaptometer. After a normal period they were put on a diet containing about 100 units vitamin A. Other vitamin supplements were given to 13, while 4 received none. No differences between the two groups appeared in rate and extent of the rise in visual threshold. In 14 subjects both rod and cone thresholds began rising at once; after a month the rod threshold had risen between 1 and 2 logarithmic units. In 3 subjects the rod threshold hardly changed during 1, 2, and 3 months respectively, after which the usual steep rise occurred.

In 9 subjects the A-deficient diet was terminated by a single ingestion of 100,000 vitamin A units. This was followed in a few hours by a drop in threshold, which was variable and never large. The threshold in no case returned to normal, or even half way to normal. In 2 cases the drop was 0.7 and 0.8 log units; in the others between 0.1 and 0.3 log units. Its extent is unrelated to vitamin supplements, or to the preceding rise.

The subsequent normal diet was supplemented in some cases with vitamin A and other vitamins. The fastest return of visual threshold to normal occurred in 40 days by two subjects; one had received no supplementary A and the other 25,000 units per day. The threshold recovery of the other subjects was slower, some not having returned to normal after 3 months.

There is thus great individual variation in the response to vitamin A deprivation, to its administration in large single doses, and to the resumption of a normal diet. These variations are probably due to conditions at present not recognized, and explain differences reported by investigators apparently repeating the same experiment.

The fate of injected radioactive chlorine. JEANNE F. MANERY. Department of Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. (Read by title.)

Radioactive chlorine was injected intraperitoneally into 5 rats and intravenously into 5 rabbits. The animals were killed by decapitation at periods from 7 to 32 minutes after injection in the case of the rats, and 11 to 52 minutes in the case of the rabbits. Plasma, urine, peritoneal fluid and tissues were ashed and analysed for chlorine by a chemical method, and for radioactivity by the Geiger-Müller counter.

The absorbed radioactive chlorine spread throughout about 30 per cent of the body weight in rats and about 22 per cent in rabbits. This and subsequent calculations are based on the assumption that chlorine exists in tissues in the same concentration as in plasma ultrafiltrate. There was no significant difference between the values obtained at 7 to 11 minutes and those found at 32-52 minutes with respect to either the percent distribution throughout the entire body or to the volumes of individual tissues penetrated by the radioactive chlorine.

The volume of tissue into which the radioactive isotope penetrated was compared to that occupied by chlorine chemically determined. These volumes approximate each other fairly closely in skin, abdominal muscle, cartilage, tendon, pyloric muscle and heart. In the gastrocnemius muscle, liver and pyloric mucosa there is some indication that the radioactive volume is less than the chemically determined chloride volume. Brain and testes are most outstanding because in both cases the volume of the radioactive space is $\frac{1}{2}$ or less than the chemical chloride space.

These results in conjunction with previously reported data on the rate of penetration of radioactive sodium into tissues throw new light on the estimation of the true extracellular space in tissues and on the extracellular position of sodium and chlorine.

The action of the right vagus upon the pacemaker of the terrapin heart. FRANK MARESH (introduced by W. J. Meek). Department of Physiology, University of Wisconsin, Madison.

After the right vagus was traced to its entrance into the walls of the right precaval vein, the sinus venosus with the attached vagus was removed from the body of the terrapin, mounted in serum upon a glass slide, and studied under the oil immersion lens of a microscope. Stimulated with tetanic current from the secondary coil of an induction coil the vagus stopped the contraction in all of the automatic fibers of the sinus venosus. Up to the present time no visible activity has been observed in the myofibrils, sarcoplasm, cross-striations or mitochondria while the vagus was exerting its effect. The right vagus stopped all contractions not only in the sinus venosus but also in the left precaval and hepatic veins as well as in the pulmonary veins. From visual observations the automatic fibers contracted simultaneously over the entire sinus venosus to form the first contraction after the vagus had stopped the heart.

Recovery cycle of the lateral geniculate of the nembutalized cat. W. H. MARSHALL and S. A. TALBOT (by invitation). Laboratory of Physiological Optics, Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital, Baltimore, Md.

Monopolar macro-electrodes were located in the optic tract, in the geniculate and on the striate cortex. Following a single shock to the optic nerve the tract shows a positive spike, the geniculate electrode records a predominately positive spike (presynaptic spike) followed at approximately 0.7 msec. by a negative spike (postsynaptic spike). The postsynaptic spike and cortical components disappear immediately when circulation fails, the presynaptic spike persists with little change for many minutes. The first and probably the second discontinuity in the cortical reaction closely follows the behavior of the post-synaptic geniculate spike. Stimulus strength was gauged by amplitude of tract spike to correct for relative refractoriness and usually prominent supernormal phases.

Using a weak conditioning stimulus followed by a test shock of comparable or much higher intensity, marked "facilitation" was found over an interval up to 20 msec. at the geniculate and cortical levels. It is prominent for conditioning shocks producing tract spikes twice the amplitude of the spike following testing shocks, if both are of low intensity. The curve of facilitation rises fast at short intervals and decreases to zero between 15 to 23 msec. It is a function of both the intensity of the conditioning and test shocks and period of stimulation cycle. The subnormal period following the facilitation interval is prominent and may be characterized by zero response at 50 to 60 msec. after the conditioning stimulus, depending on the intensity of conditioning shocks. A brief volley of weak shocks may prolong the period of minimum or zero response from 24 msec. to 60 msec. As the conditioning shock intensity is increased the facilitation interval may decrease, complete recovery may require one to four seconds.

Application of conditioning shock to one optic nerve and test shock to the other has revealed comparable interaction only at cortical levels.

This pattern of behavior helps to account for the relatively large "on" response following weak sensory stimuli.

Action potential response of the dorsal surfaces of the cat's cortex elicited by brief and approximately total stimulation of the retina. WADE H. MARSHALL, S. A. TALBOT (by invitation) and H. W. ADES (by invitation). Laboratory of Physiological Optics, Wilmer Ophthalmological Institute, Johns Hopkins University and Hospital, Baltimore, Md. (Read by title.)

The responses are of highest amplitude and are typically multiple over the marginal gyrus. Rostrally they decrease to zero at a point approximately determined by a line joining the anterior inflexions of the sulci suprasylvii. Caudally the amplitude decreases in the region just anterior to the point where the gyrus marginalis curves into the compositus. The entire gyrus compositus is characterized by a response of smaller amplitude and shorter duration of the positive phase. These features are approximately similar under deep nembutal, light nembutal, and ether anesthesia. There appears to be a good correlation between the amplitude of the surface positive wave and the population density of the larger cells of the upper pyramidal layer.

In deep nembutal anesthesia the striate area responses are nearly monophasic and surface positive; in light anesthesia they are typically diphasic with positive phase leading. Localized application of a convulsant drug (picrotoxin) produces some enhancement of the positive phase and great

enhancement of the negative phase. The positive phase is very resistant to depressants.

Responses are always found in various foci over the middle and posterior suprasylvian gyrus. They consist of two components. One has many characteristics of a primary projection response and in one area over the middle region of the suprasylvian just bordering on the sulcus suprasylvius the response is still present after removal of the entire striate, posterior suprasylvian and section in the thalamus medial to the geniculate. The second component can be elicited or increased by local application of picrotoxin on an appropriate area of the striate cortex, an effect first observed by Curtis and Bard on the somatic areas. Local application of a depressant (nembutal) on the striate spot previously activated by picrotoxin abolishes the negative wave on the striate and the positive wave on the suprasylvian.

The action of Welch-like bacilli in the liver. EDWARD C. MASON. Department of Physiology, University of Oklahoma School of Medicine, Oklahoma City.

If a small piece of liver is sectioned aseptically and dropped into the abdominal cavity of a dog or rabbit, death occurs in fifteen to eighteen hours. Mason and Davidson published the first recorded observation on this subject in 1925 and were of the opinion that death was due to liver autolysis. The most marked changes occur in the free liver tissue; it undergoes a complete transformation. The color becomes that of light chocolate and the feel that of lung tissue, being spongy and gas containing. In our early observations we assumed that the presence of gas was due to the action of the colon bacillus; however, subsequently it was shown to be produced by a Welch-like bacillus.

Death appears to be due to products of autolysis and the action of the bacillus on dead adult liver tissue. The organism administered either intraperitoneally or intravenously has no apparent effect on the intact animal. The chemical peritonitis resulting from the presence of the sectioned liver increases the permeability of the abdominal viscera and fluid accumulates in the abdomen with a resulting blood concentration, producing the picture of shock. Fluids including water and solutions of sugar, salt or gum, given postoperatively by the various channels, does not alter the length of life.

In our present study we have cultured human liver tissue and have repeatedly found a Welch-like bacillus. We have called this organism the Welch-like bacillus due to the fact it has the following characteristics in common with the *B. welchii*: 1, it grows under strict anaerobic conditions; 2, is gas forming, liberating seven to ten volumes of gas from liver tissue; 3, is gram-positive; 4, produces "stormy fermentation of milk," and 5, gives the Welch-Nuttall rabbit test. These tests are presumptive tests for *B. welchii*, they are not conclusive but strongly suggestive of the presence of the organism in the material under investigation.

It is conceivable that injuries to the human liver which cause impairment of circulation may afford a culture medium for these organisms and thus cause death.

Variations in hemoglobin concentrations in the same individual. M. E. MAXFIELD (introduced by H. C. Bazett). Department of Physiology, University of Pennsylvania, Philadelphia.

Hemoglobin concentrations were determined by a method based on the principles of Hartmann (see Demonstration, M. E. Maxfield et al). As determined by comparisons of duplicate dilutions of the same blood the method permits an over-all accuracy with a standard deviation of ± 0.23 per cent from the mean of the pairs. Errors associated with the withdrawal of blood are unlikely to exceed ± 1.1 per cent since the mean difference between two samples drawn at an interval of 20 to 25 minutes has been of this order (22 comparisons) even though the subject has been inhaling CO and O₂ during the interval. In one subject studied on several occasions there has been a consistent progressive increase in hemoglobin concentration during the first 2 to 3 hours after waking. The maximum change under basal conditions has been only 3.7 per cent. When a meal was taken it reached 4.7 per cent.

We would like to thank the John and Mary R. Markle Foundation whose support made this work possible.

A differential photoelectric colorimeter for determination of hemoglobin concentration and of small percentages of carbon-monoxide hemoglobin. M. E. MAXFIELD (by invitation), H. C. BAZETT, C. C. CHAMBERS (by invitation) and C. KELLY (by invitation). Department of Physiology, University of Pennsylvania, Philadelphia. (Demonstration.)

The apparatus was designed to measure blood volume according to the principles described by Hartmann (Ergeb. Physiol. 39: 413, 1937). It consists of a differential photoelectric circuit with an "electric eye" as a null-point indicator (after Shepard, RCA Review 2: 160, 1937). Balance is attained by moving the light along a 40 inch optical bench. No electrical adjustments are made. A high pressure mercury vapor lamp is used with filters for the 546 m μ and 578 m μ lines (for green Wratten 62 + Corning 5.1 combination and for yellow Zeiss A combination + 1 mm. Jena BG 7). A 12 mm. thickness of 0.35 M CuSO₄ is used in addition at both wave lengths. Holders for the two filters and another carrying 3 cells (2 bloods and 1 blank) rotate on pivots controlled by levers. A standard gray glass can be inserted behind the control cell. Cells of 0.6 to 5.0 mm. thickness requiring volumes of 0.3 to 3.0 ml. are utilized. The position of the zero point is adjustable by means of an iris diaphragm in front of the control photocell.

The caesium cells used are 917 and 919 both connected to the grid of a single tube amplifier. Alternating current (115 v) from the lighting circuit is utilized with stabilizers (Sola) on both the lamp and amplifier circuits. Fair accuracy is attainable without stabilizers and some of the data cited were so obtained. Normally 0.4 ml. of blood diluted to 11 ml. for hemolysis was used in 1 mm. cells. Total circulating hemoglobin was estimated by the inhalation of 100 ml. of CO which gave a CO saturation of 7.5 to 8.9 per cent. Duplicate estimates were made for hemoglobin concentration (15) and also for total hemoglobin (5). The standard deviations of these estimates from their means were respectively ± 0.23 per cent and ± 2.1 per cent.

The apparatus can also be adapted for other precise colorimeter measurements.

We are indebted to the Markle Foundation for the support of this work

*The influence of posture on blood flow in the dog.*¹ H. S. MAYERSON. Laboratory of Physiology, Tulane University School of Medicine, New Orleans, La.

Blood flow was measured by means of thermostromuhr units in dogs anesthetized with chloralose or sodium barbital when they were moved from the horizontal to the upright (75°) position, feet down, on a tilting table. Immediately after tilting, the rate of flow in the carotid artery and jugular vein was usually diminished while that in the femoral artery and vein was increased. As the upright position was maintained, the rate of flow in the carotid artery and jugular vein in some instances showed a secondary rise lasting from 3 to 4 minutes. At the end of 10 minutes in the upright position, however, the rate of flow in these vessels in all cases was from 10 to 50 per cent lower than before the tilting. The increased rate of flow in the femoral artery and vein likewise persisted for only 3 to 4 minutes, after which time it diminished, so that at the end of 10 minutes the rate of flow in the artery was 16 to 50 per cent less than it was before tilting and 36 to 88 per cent lower in the vein. In several experiments the flow in the vein was almost stopped at the end of this period. The rate of flow in the renal artery, in several cases, showed no significant change when the animal was in the upright position, in other experiments the rate was decreased (maximum decrease = 50 per cent). The flow in the renal vein, however, was always diminished 30 to 40 per cent below the control value. In one experiment, it decreased from 124 cc. per minute to 5 cc. per minute at the end of 10 minutes in the upright position. In the majority of experiments the rates of flow in all the vessels approximated the pre-tilting values within 6 to 10 minutes after the animals were returned to the horizontal position. In some instances the flow immediately after the animal was returned was considerably higher than the control value, dropping gradually to the pretilting level after 3 to 4 minutes.

*Functional interdependence of sensory cortex and thalamus.*² W. S. McCULLOCH and J. G. DUSSEY DE BARENNE. Laboratory of Neurophysiology, Yale University, School of Medicine, New Haven, Conn.

The electrocorticogram is normal in the decerebrate monkey. It is permanently abnormal in the undercut cortex. The electrogram of the thalamic sensory nuclei severed from their cerebral cortex is permanently abnormal. These data show that the cortex and thalamus are functionally interdependent.

*Studies on auriculo-ventricular conductivity.*³ R. S. MEGIBOW (by invitation) and L. N. KATZ. Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

The effects of several drugs on auriculo-ventricular conductivity were studied by the method previously described (Carlen and Katz, *Am. J. Physiol.* 127: 272, 1939). An attempt was also made to analyze the mode of action of asphyxia on this property. Seventeen dogs anesthetized with nembutal were used.

¹ Aided by a grant from the David Trautman Schwartz Research Fund of the Tulane University School of Medicine.

² Aided by a grant from the Fluid Research Funds of the Yale School of Medicine.

³ Aided by the A. D. Nast Fund for Cardiac Research.

Paredrine hydrobromide (20 mgm.) caused a progressive increase in A-V conductivity in both the denervated and innervated heart. Barium chloride (50 mgm.) caused a marked enhancement of A-V conductivity in the denervated animal and a slight increase in A-V conductivity in the innervated heart. Atropine sulfate (2 to 2.5 mgm.) caused a marked enhancement of A-V conductivity in the innervated heart but a moderate depression in the denervated heart. Quinidine sulfate (30 to 60 mgm.) caused a rapid and marked depression of A-V conductivity in the denervated animal, leading in one dog to complete A-V block. Sodium bicarbonate (1 gram) caused a marked initial depression followed rapidly by an increase in A-V conductivity in both the denervated and innervated heart. Graded concentrations of CO_2 in O_2 in inspired air varying from 5 per cent to 30 per cent was used in both the innervated and denervated animal. The results were similar in both, although more marked in the denervated heart; they consisted in an increased A-V conductivity at concentrations of 5 to 12½ per cent CO_2 , and a gradual progressive depression going on to complete heart block when the CO_2 concentration was increased above this. Asphyxia, produced by stopping artificial respiration and anoxemia, produced by delivering nitrogen in place of air, also had a diphasic action in the innervated, the denervated and the eviscerated animal. In all preparations, asphyxia and anoxemia enhanced A-V conductivity in the early stages, and depressed conduction in the more advanced stages. In asphyxia, on resumption of respiration a period of enhanced A-V conduction appeared before normal conductivity was regained.

The diphasic changes in asphyxia are therefore partly due to hypercapnia and partly to anoxemia. This diphasic action can be attributed almost entirely to a direct action on the heart; reflex and distant humoral actions playing at most only a minor rôle.

The influence of cerebrospinal fluid calcium on a spinal flexion reflex. JEROME

K. MERLIS (introduced by Hampden Lawson). Department of Physiology, University of Louisville School of Medicine, Louisville, Ky.

The spinal tibialis anticus reflex in barbitalized dogs was elicited while perfusing the spinal subarachnoid space with solutions containing varying amounts of calcium. The perfusion was limited to the subarachnoid space below the level of transection (T 10) and was maintained at constant pressure (below mean carotid pressure) and temperature according to the method of Merlis and Lawson (J. Neurophysiol. 2: 566, 1939). An artificial cerebrospinal fluid was used in control perfusions, containing the following in moles per liter: Cl-0.152, HCO^- -0.921, HPO_4^{2-} -0.00048, Na-0.141, K-0.0033, Ca-0.00125, Mg-0.0012, glucose-0.0034, urea-0.0022. When the calcium concentration was changed, the solution was made isosmolar by changing the NaCl concentration an equivalent amount.

In a few animals a comparison of the tibialis anticus reflex before and after beginning perfusion with the balanced artificial spinal fluid was made. In these, substitution of the artificial for the natural spinal fluid was without apparent effect. No progressive change in the reflex was observed during perfusion with the balanced fluid for several hours. On substitution of an artificial fluid containing no calcium, there was augmentation of the reflex, and an increase in the tone of the muscle. During this augmentation, spontaneous twitching of the muscles of the lower half of the body was seen. On replacing this with the balanced fluid there

was a prompt subsidence of all these effects. Solutions containing an excess of calcium (up to 4 times the concentration of the balanced solution) had no effect on the reflex. The intensity of the effects from calcium-free solutions appeared to be proportional to the rate at which the fluids passed through the subarachnoid space.

Extrapyramidal inhibition of cortically induced movement. FRED A. METTLER and CECILIA C. METTLER (by invitation). Child Neurology Research (Friedsam Foundation), Department of Anatomy, University of Georgia School of Medicine, Augusta.

We have previously reported upon the inhibiting effect of stimulation of the caudate and putamen upon cortically induced movements. (51st Annual Meeting of the Society, see p. 168 of Proceedings for 1939 and also Arch. Neur. and Psychiat. 41: 984, 1939).

The present research deals with an exploration of various portions of the caudate in an attempt to identify any systematic localization pattern which may be present.

Numerous experiments directed toward this end failed to show any such somatotopical pattern. It was, however, discovered that the intensity of the inhibitory effect varies from place to place within the caudate. On the whole, the mediodorsal aspect of the nucleus is more effective than other portions and that part situated on a level with the curvature of the fornix more active in this respect than more anterior or posterior portions.

Examination of sections of these regions shows that the most susceptible parts of the caudate coincide with the greatest concentration of subcallosal fasciculus fibers. We have previously (J. Comp. Neur. LXI, 509-42, 1935) presented evidence in favor of the view that this bundle consists, in part at least, of corticocaudate fibers and the susceptibility of this region to stimulation would seem to imply that stimulation of the subcallosal fasciculus results in simultaneous irradiation of practically all of the caudate. Since it seems improbable that it would be possible to avoid this system of fibers during stereotaxic stimulation without previous degeneration of the subcallosal fasciculus we are in no position to say whether or not a basic pattern of somatotopical localization exists in the caudate nucleus.

*The rôle of afferent impulses in the response to cortical stimulation.*¹ FRED A. METTLER, C. G. SMITH (by invitation) and E. A. CULLER. Departments of Anatomy, University of Georgia School of Medicine, University of Toronto and Department of Psychology, University of Rochester. (Motion picture demonstration.)

At the 51st annual meeting of this society (see Proceedings, p. 168) Mettler, Ades, Lipman and Culler reported upon the method of multiple stimulation as applied to the study of the corpus striatum. The basic movement utilized in this study was the phasic reaction obtained by stimulation of the motor cortex.

The phasic or alternating manifestation of a constant stimulus flow in cortical excitation implies the interpolation of an interruptor mechanism which the present research was designed to elucidate.

¹ Financed by Child Neurology Research.

It was found that cutting the dorsal roots of the segments involved in such a phasic motor response resulted in its conversion into a tonic response. Stimulation of the motor cortex following severance of all the muscles but one innervated by the brachial plexus results in phasic contractions of this single muscle so that at least some of the afferent impulses involved in the phasic response originate as proprioceptive stimuli within the contracting muscle itself.

The question now arises as to whether the interruption of the steady neural flow involved in the tonic response occurs at the segmental or higher levels. Severance of the dorsal columns does not convert phasic into tonic activity. Severance of that part of the cord in which the spinocerebellar tracts are located does (the ventral spinocerebellar tract deals with the forelimb). See also abstract of paper by Fred A. and Cecilia C. Mettler, this publication.

An electrodynamic method for the quantitative extraction of histamine from small quantities of blood and plasma. DAVID MINARD (introduced by G. E. WAKERLIN). Department of Physiology, College of Medicine, University of Illinois, Chicago.

Present methods for the extraction of histamine from blood require relatively large quantities of blood which is subjected to severe chemical treatment, thus suggesting that the extracted histamine may result from chemical decomposition.

Electrodynamic extraction of histamine meets the latter objection as well as being a more rapid and less tedious method. MacGregor and Thorpe's procedure of electrodynamic extraction (Biochem. J. 27: 1394, 1933) has been employed successfully by us in quantitatively extracting histamine from rabbit blood, in which the histamine content is relatively high, thus enabling electrodynamic extraction of a small volume of blood into a large volume of water.

Attempts either to demonstrate the presence of histamine in normal blood or plasma of low histamine content (dog, human) or to recover added histamine were unsuccessful when electrodynamic extraction of a small volume of blood against an equal volume of water. The naturally occurring histamine, as well as added histamine, was found to be destroyed by the high alkalinity of the cathode fluid. Under these conditions, however, quantitative extraction can be achieved if the change in pH of the cathode fluid is buffered by bubbling CO₂ through the fluid during electrodynamic extraction.

The three chambered cells employed in our method, though essentially similar in design to those described by MacGregor and Thorpe, differ in the following respects: Each chamber has a capacity of only 2 cc.; the electrode chambers are milled from solid blocks of Lucite; the middle chamber is cut from pure gum rubber sheet, the chambers being separated by cellophane membranes; the electrodes are of platinum mesh; B batteries with series resistance serve as the current source; cooling coils are used in the middle chambers only.

After completion of the electrodynamic extraction the cathode fluid is neutralized (driving off the combined CO₂) with N HCl against neutral red and tested directly on the isolated atropinized guinea pig intestine.

The active constituent in the cathode fluid following electrodynamic extraction of dog blood and plasma and of human plasma is histamine-like in character,

the histamine equivalent values agreeing closely with those obtained by the method of Barsoum and Gaddum.

Studies on carbohydrate and fat metabolism of normal and depancreatized ducks. I. ARTHUR MIRSKY, NORTON NELSON (by invitation) and ISABELLE GRAYMAN (by invitation). The May Institute for Medical Research of the Jewish Hospital, Cincinnati, O.

It has been known for many years that the removal of the pancreas from the fowl does not result in any significant change in the blood sugar level. Likewise no change from the normal has been observed with respect to liver glycogen or the response to exogenous insulin (Ivy).

The present report summarizes our observations concerning several aspects of carbohydrate and fat metabolism in the duck subjected to fasting periods of at least 30 days.

1. Whereas the normal dog or cat shows an insignificant ketonemia (less than 15 mgm. per cent) during a fast of 30 days, the normal duck develops a relatively high ketonemia (av. 60 mgm. per cent).

2. Removal of the pancreas intensifies the fasting ketosis of the dog (up to 40 mgm. per cent) and cat, but tends to depress that of the duck (av. 30 mgm. per cent).

3. The administration of glucose intravenously diminishes the ketonemia in the fasted duck. The fed depancreatized duck likewise shows no significant ketonemia. The tissues of the duck can utilize exogenous acetone bodies.

4. The daily administration of phlorhizin to the fasting dog induces a much more severe ketosis in the absence of the pancreas than in its presence. This is not the case in the duck where phlorhizin produces an extremely high ketonemia in both normal and depancreatized states (over 100 mgm. per cent).

5. Whereas the administration of phlorhizin induces a hypoglycemia in the normal or depancreatized dog, it has no apparent influence on the blood sugar of the normal or depancreatized duck in spite of the fact that a severe ketosis and glycosuria ensue.

The significance of these observations together with others on the dextrose tolerance curve and nitrogen excretion of the normal and depancreatized duck will be discussed.

Cardiac and pulmonary edema in isolated perfused preparations. G. K. MOE and E. H. WOOD (introduced by M. B. Visscher). University of Minnesota, Minneapolis.

Studies of concentration changes of constituents of perfused heart muscle have failed to correct properly for weight changes resulting from accumulation of edema fluid. Simple determination of the water content of the tissue, often used as a criterion of edema, is obviously insufficient, since the edema fluid may have a dry weight exceeding that of the plasma if corpuscles accumulate.

Calculation of edema from chloride analyses ("chloride space"; Hastings, Fenn) is based on two assumptions: that the muscle cell retains its normal impermeability to chloride and that all of the edema is extracellular. To check the accuracy of the chloride space method, four experiments were done on Langendorff dog heart preparations. These hearts were suspended from a pulley and balance arrangement which permitted a contin-

uous record of weight changes of the heart. Cardiac edema, determined as the difference between initial and final weight, was compared with edema determined by the chloride space method. The average deviation between the two methods was 2.5 per cent. Permeability of dead muscle tissue to chloride should cause a positive error in the chloride space calculation, but intracellular edema, which is not included in edema calculated from chloride analyses, would tend to cancel this error.

In heart-lung preparations edema of both organs presumably results from similar causes; a correlation was therefore sought between cardiac edema (chloride method) and lung edema (weight measurement). The relative values correlate closely, giving a straight line along which 10 per cent increase in lung weight coincides with 3.7 per cent increase in cardiac mass. This relation therefore gives a convenient and simple means of roughly estimating cardiac edema from lung weights. This method has been checked by determining ventricular weight/body weight ratios for 85 heart-lung hearts before and after correction for edema. The results are summarized below:

	NORMAL HEARTS		HEART LUNG HEARTS	
Number	10	200*	85	After correction for edema
(V.W./B.W.)100	0.706	0.709	0.920	0.715

* From G. R. Herrmann.

The plasma protein content of the reservoir blood of the spleen. A. G. MULDER, FRANK TULLIS, JR. (by invitation) and MARGARET MAYNARD (by invitation). Department of Physiology, College of Medicine, University of Tennessee, Memphis.

In these experiments an attempt was made to determine whether the spleen concentrated plasma proteins as well as red blood cells. After the withdrawal of samples of blood from the femoral vein and the splenic vein the coeliac axis was tightly clamped, a cannula was placed in the splenic vein, and the nerves to the spleen were stimulated. All blood samples were analysed for their plasma protein content.

In all of our eight experiments the plasma protein content of the blood freely flowing through the splenic vein showed either the same or a lesser concentration of plasma protein when compared with that of the femoral vein.

The plasma protein content of the blood obtained as a result of the splenic contraction was variable. The red cell concentration in the reservoir blood was always high.

A comparison of the polarographic apparent oxidation potential of ascorbic acid and the oxidation-reduction potential of the system ascorbic acid-dehydroascorbic acid. OTTO H. MÜLLER (by invitation) and R. A. PHILLIPS. Departments of Surgery and Physiology, New York Hospital and Cornell University Medical College, New York City.

The oxidation of ascorbic acid at the dropping mercury electrode has been studied over the range of pH 1 to pH 13. Above pH 8 there is evidence that the first oxidation product is further oxidized at the dropping mercury electrode. A graph of the polarographic apparent oxidation potential (Müller Chem. Rev. 24: 95, 1939) of the first step of oxidation

plotted against pH shows only one inflection around pH 4.2. The P.A.O.P.-pH curve of the second step of oxidation shows an inflection near pH 11. The P.A.O.P. values of the first step are consistently more positive (by about 160 mv.) than the oxidation-reduction potentials of the ascorbic acid—dehydroascorbic acid system. However, this system is known to be sluggish because the potentials drift towards plateau values unless a mediator is added to accelerate the establishment of potential.

To investigate this further, some of Ball's experiments (J. Biol. Chem. **118**: 219, 1937) were repeated and the solutions were analyzed potentiometrically and polarographically at different stages of the titration. Potentials in good agreement with Ball's results were found when the dropping mercury electrode was used as indicator electrode. An analysis of the polarographic curves, however, showed that whatever poisoning substance was present must have been extremely dilute since no measurable wave due to the reduction of the dehydroascorbic acid appeared on the polarogram.

Studies on the absorption of pectic and uronic acids. HARVEY K. MURER (by invitation) and LATHAN A. CRANDALL, JR. Department of Physiology, University of Tennessee, Memphis.

Since the fate of fed uronic acid compounds is unknown, their convertibility into glucose was determined by oral administration to phlorizinized dogs. Pectic acid or calcium galacturonate was given orally in doses of 20 to 25 grams. Glucose and nitrogen were determined quantitatively in the urine, and urinary uronic acids were measured by a semi-quantitative method. Neither pectic nor galacturonic acid gave rise to an extra excretion of glucose; the D/N ratio did not increase, and the amount of dextrose and nitrogen excreted per day remained unchanged. Following oral administration of calcium galacturonate a small fraction of the uronic acid (not more than 1 gram) was excreted in the urine during the next 48 hours. It is concluded that galacturonic acid does not serve as a glucose precursor.

Normal dogs were given 20 grams of pectin or pectic acid by mouth and the amount of pectic acid excreted in the stool was determined. The greater part of the administered pectin appeared in the stool unchanged. It seems probable that pectin is not hydrolyzed in the digestive tract of the dog. Galacturonic acid when given as such is presumably absorbed and the greater part metabolized without being converted to glucose.

Studies on cholinesterase from the electric organ of torpedo. D. NACHMAN-SOHN (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.¹

The concentration of cholinesterase, strong everywhere in nervous tissue, rises to high values at all ganglionic and central synapses and neuromuscular junctions. The concentration is high enough to split in milliseconds amounts of ACh which have a stimulating action on the effector cells. This suggests that the physiological significance of the enzyme might be the removal of ACh during the refractory period required by the theory that ACh might be connected with transmission of nerve impulses at these regions.

¹ This investigation was aided by a grant from the Dazian Foundation.

The high concentration of the enzyme at muscle endplates suggested that electric organs, considered as a collection of modified endplates, might have a similar mechanism. It was found that the enzyme concentration in the electric organ of *Torpedo* is of the same order of magnitude as at muscle endplates and higher than in any other organ. The organ was therefore used, with Lederer, as material for the preparation of active enzyme solutions with which some properties of the enzyme were investigated.

An organ of medium size splits about 100 mg. ACh in 1 sec. Such powerful enzymatic mechanism suggests that its function is as elsewhere the quick removal of liberated ACh. Recent investigations, with Feldberg and Fessard, brought evidence that nerve stimulation liberates ACh in the organ and that small amounts of ACh injected produce a discharge. These experiments emphasize the physiological significance of the enzyme and make it desirable to study its properties in more detail. Some physicochemical constants are being determined, e.g., the temperature coefficient, influence of pH, action of different ions, especially heavy metals, and of some alcaloids, which are of interest in connection with the transmitter problem.

Production of auricular fibrillation by application of acetyl- β -methylcholine chloride to localized regions on the auricular surface. L. H. NAHUM and H. E. HOFF. Laboratory of Physiology, Yale University School of Medicine, New Haven.¹

Other workers have produced auricular fibrillation in dogs by stimulation by the vagus nerve or by injection of acetylcholine. We have elsewhere reported the production of auricular fibrillation by injection of acetyl- β -methylcholine chloride in hyperthyroid patients and by electric shock in cats given the same substance. In the experiments reported here application of this drug to the surface of the auricles on small strips of filter paper (0.2 x 1.0 cm. in the dog) in concentrations of 1:500 to 1:2000 has led in some instances to the onset of auricular fibrillation without any further procedures. When the arrhythmia failed to develop following application of the drug alone, it could always be evoked by mechanical stimulation of any part of the auricles. In one experiment auricular fibrillation was provoked by mechanical stimulation after application of the drug to any region of either auricle, but in most animals application over the sino-auricular node was required. The experiment succeeded in both cats and dogs. Fibrillation persisted for three to five minutes after removal of the pledget, and for one to two minutes thereafter could be restarted for a few seconds by new stimulation. Thereafter new treatment with acetyl- β -methylcholine was required to reproduce the arrhythmia.

Application of the drug over the pacemaker caused slowing of the whole heart, and arrest of the auricles within half a minute. During this period, and for several minutes after recovery of auricular beats, mechanical stimulation precipitated the arrhythmia. When auricular fibrillation occurred without mechanical stimulation, it followed one of the first few auricular beats.

Local application of atropine prevented all effects of the drug when applied to the same region, but not when some other region was treated. Atropine did not affect the arrhythmia once it had been produced.

¹ This investigation was aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

Effect of hypoglycemia on gastric motility. H. NECHELES, WM. OLSON (by invitation) and ROBERT MORRIS (by invitation). Department of Gastro-Intestinal Research, Michael Reese Hospital, and Department of Physiology, University of Chicago, Chicago, Ill.

It is well known that insulin hypoglycemia is accompanied by increased gastric motility. We have analyzed this phenomenon in unanesthetized trained dogs with gastrostomies employing the usual technique of balloon with manometer recording. Insulin was injected subcutaneously, 0.65 unit per kilo of body weight. In a number of dogs the blood sugar fell to extremely low levels, but no convulsions appeared. At this stage gastric motility was depressed or absent. As the blood sugar rose spontaneously or by injection of glucose (to 30 mgm. and above) strong continuous motility followed and continued until blood sugar approximated normal levels. At this time regular periodic hunger motility appeared.

Similar observations have been reported on humans, but not to our knowledge on dogs. We may be dealing with a depression of gastric motility due to either preconvulsive state and nausea or reversal or drug action on the vagus center.

The non-effectiveness of riboflavin phosphoric acid and riboflavin in maintaining life in the adrenalectomized rat. DOROTHY NELSON (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill. (Read by title.)

Riboflavin phosphoric acid in daily doses of 30 γ to 120 γ is unable to sustain life in bilaterally adrenalectomized rats. A comparison has been made of the survival time of animals treated with riboflavin phosphoric acid with that of others given unesterified riboflavin, and with untreated adrenalectomized controls.

Of the 360 bilaterally adrenalectomized rats, 58 (16 per cent) were not included in the comparison because of indefinite survival due to regeneration of adrenal tissue, or because of premature death caused by operative trauma or mechanical injury in the cages. Riboflavin phosphoric acid was given to 93 animals, riboflavin to 73, and 136 were not treated. Tap water and a routine laboratory diet was given to all animals with no salt supplement.

Statistical analysis of the data showed no significant differences between the three groups. The average survival time of the riboflavin phosphoric acid group was 7.62 ± 0.36 days, that of the riboflavin group was 7.32 ± 0.40 days, and that of the untreated controls 7.56 ± 0.32 days.

Daily doses of 30, 60 or 120 γ of riboflavin phosphoric acid were administered. Riboflavin was given orally in daily doses of 400 γ or 800 γ . Variation in the size of the dose did not significantly modify the response.

These findings indicate that the vital function of the adrenal cortex is not the phosphorylation of riboflavin in the synthesis of the yellow enzyme.

The influence of glomerular filtration on the glucose "threshold" in man.

NORTON NELSON (by invitation) and I. ARTHUR MIRSKY. The May Institute for Medical Research of the Jewish Hospital, Cincinnati, O.

The recent studies of Fisher and Shannon with dogs make it probable that the so-called renal "threshold" for glucose is dependent upon the reabsorption of a constant amount of sugar from the glomerular filtrate and not upon the concentration of sugar in the filtrate. On the basis of this

concept, a reduction in the rate of glomerular filtration would require a higher concentration of glucose in the filtrate in order that the maximum reabsorptive capacity of the tubules might be attained. This concentration of glucose thus becomes the so-called renal "threshold" for glucose.

In order to put the above to test in man, subjects with various degrees of glomerular damage were studied and both urea and glucose clearances determined. From these clearances and the assumption that the urea clearance bears a constant relationship to glomerular filtration ($\frac{\text{urea}}{\text{inulin}} = 0.6$) the rate of sugar reabsorption was calculated. The mgm. of sugar reabsorbed per 100 cc. of glomerular filtrate thus indicated the numeric "threshold."

Our data reveal that when the urea clearance is markedly impaired in the presence of an insignificant depression of sugar reabsorption by the tubules, the numeric "threshold" rises (e.g., elderly diabetics). On the other hand, when the sugar reabsorption is diminished while the glomerular filtration remains unimpaired, the numeric "threshold" falls (renal glycosuria). Our studies in man support the concept that the "threshold" is dependent upon the capacity of the tubules to reabsorb a definite amount of sugar and not upon the concentration of sugar in the blood stream.

Maintenance of spermatogenesis in hypophysectomized rats. WARREN O. NELSON. Department of Anatomy, Wayne University, College of Medicine, Detroit, Mich. (Read by title.)

In earlier experiments we have reported the maintenance of spermatogenesis in hypophysectomized rats treated with androgenic hormone for as long as 60 days. These studies have been continued and it has been observed that spermatogenesis may be maintained for at least 178 days. Testosterone-propionate and androstanedione in doses of 2 mg. daily have been used in these studies.

The following is a summary of the periods of treatment, dating from the time of hypophysectomy, and the number of animals in each group: 90 days (3 treated, 2 untreated), 125 days (4 treated, 2 untreated), 155 days (3 treated, 3 untreated), 178 days (3 treated, 2 untreated). The testes of all treated animals showed active spermatogenesis and the epididymidi contained motile sperm. Females in estrus were presented, at intervals, to each male. In the majority of instances copulation took place and viable litters were born. Thus litters were sired as long as 169 days after hypophysectomy.

Although the testes became progressively smaller as treatment was continued, they far exceeded the testes of untreated controls, e.g., the average weight of the testes of the 178 day series was 1.609 gram for the treated animals, while the testes of the untreated controls was 0.273 gram. These experiments leave little reason to doubt that activity of the germinal epithelium can be continued in the absence of the hypophysis in animals treated with androgenic hormone.

Re-initiation of spermatogenesis in hypophysectomized rats. WARREN O. NELSON. Department of Anatomy, Wayne University, College of Medicine, Detroit, Mich. (Read by title.)

In earlier papers we have reported that spermatogenesis can be saved

in the testes of rats if treatment with androgenic hormone is begun within 10 days after hypophysectomy. If the androgen is withheld for longer periods the testis is unaffected by the treatment. Continued study of this problem has revealed additional evidence some of which is reported at this time.

Animals treated immediately after hypophysectomy with adrenal cortical extract show diminished testicular atrophy and with some preparations spermatogenesis has been maintained for 20 days. The sex accessory glands of these animals have shown evidence of slight, but unmistakable androgenic stimulation. With other preparations of cortical hormone the sex accessories have shown exceedingly little or no evidence of androgenic stimulation, and the testes have shown approximately the degree of atrophy seen regularly 20 days after hypophysectomy. However, in such animals the subsequent injection of androgen (testosterone-propionate or androstane-dione) has resulted in an increase in testicular size and a return of spermatogenesis. In these experiments the animals were hypophysectomized and treatments with cortical hormone (1 to 2 cc. daily) were begun at once. Twenty days later each animal was operated and one testis and epididymis, and one seminal vesicle were removed, weighed and prepared for study. Uniformly, the testes have shown absence of active spermatogenesis, the epididymidi absence of sperm, and the seminal vesicles have been atrophied. Following the removal of these tissues the animals were treated with 2 mg. daily of androgen, in addition to the cortical hormone, for 20 to 25 days. At the end of this period the animals were sacrificed and the remaining tissues removed for study. In each instance (10 animals) the remaining testis has been larger than the one removed at operation and in 8 cases the testes have shown a re-initiation of spermatogenesis and the epididymidi the presence of sperm. Motility of sperm has varied to a considerable degree.

Further studies in which the combinations of substances used in the treatment and the periods of treatment are being varied are in progress.

A comparison of the systolic blood pressure in the right and left arms of medical students. L. B. NICE, G. F. P. EHLERS (by invitation) and F. B. WARNER (by invitation). The Chicago Medical School, Chicago, Ill. (Read by title.)

We have measured the systolic and diastolic blood pressures in the quiet (basal) supine position by means of a mercury manometer in 104 Sophomore medical students. In one class ranging from 21 to 30 years, 19 out of 50 had a systolic blood pressure ranging 3 to 12 mm. Hg higher in the right than in the left arm, while 5 out of 50 had a pressure in the left arm higher than in the right arm. In the second class of 54 ranging in years from 21 to 40, the systolic blood pressure in the right arm was higher than in the left in 19 out of 54 while it was higher in the left arm in 5 out of the 54 cases. Thus 38 of these students out of 104 showed a systolic pressure higher in the right arm than in the left while 10 had a pressure in the left arm higher than in the right. Lewis' patients ranging from 40 to 101 years of age showed a higher blood pressure in the right than in the left arm in 63 out of 103 cases while it was higher in the left arm in 13 out of 103 cases (Am. J. Physiol. 121: 517, 1938).

The response of surviving strips of cecum to changes in its environment. PAUL A. NICOLL (introduced by A. J. Carlson). Department of Physiology, University of Chicago, Chicago, Ill.

From the cecum, especially of the guinea pig, strips of smooth muscle consisting of the circular layer only are readily obtained. Such strips show under suitable environmental conditions rhythmical beating movements that will continue for days. The preparations were studied in a modified muscle bath described in detail elsewhere.

In the original observations a modified Lock's solution was used with air bubbled through to oxygenate and stir the medium. Under these conditions the strip assumes a moderate tone upon which is superimposed the rhythmical beats. The solution with a $[\text{NaHCO}_3]$ of 11 mM/L reached at equilibrium a pH value of approximately 8.3. The exact value depended of course on the exact $[\text{NaHCO}_3]$ and the partial pressure of CO_2 in the air. If now in place of air a gas mixture of approximately 95 per cent O_2 and 5 per cent CO_2 was substituted the pH value fell rapidly to approximately 6.6 at equilibrium. Within a few seconds after the change of gases and before equilibrium is reached the tissue shows an initial stimulation both in tone and amplitude of the beats. This is followed rapidly by complete loss of tonus and absolute quiescence. However, upon continuation under these conditions the tissue after varying intervals of time suddenly shows a burst of rhythmical beats usually accompanied by a rise in tonus level. This condition will continue for an indefinite time. Upon reversal to air a series of changes in the reverse order are obtained.

The tissue response can not be directly correlated with change in the pH of the medium alone. In experiments using carefully standardized salt solutions with a $[\text{NaHCO}_3]$ of approximately 25 mM/L, the tissues show moderate tonus and rhythmical beats when the gas mixture contains approximately 7 per cent CO_2 . However, changes in the CO_2 content of the gas mixture in either direction, leading to a decrease or increase of pH as the case may be, produces a fall in tonus and complete quiescence. The effect is reversible under all conditions studied.

The distribution of artificial radioactive potassium in the tissues of rats at various times after injection. T. R. NOONAN (by invitation), W. O. FENN and L. F. HAEGE (by invitation). The University of Rochester, Rochester, N. Y.

Radioactive potassium has been administered to rats by stomach tube and by intraperitoneal or subcutaneous injection. After injection, the animals were killed at intervals varying from 30 minutes to 15 hours. Different tissues were sampled for analysis and determinations were made in plasma, cells, tissues and the carcass as a whole, of the ratio, R, between the radioactive K (in per cent of the amount injected per Kg) and the total K (in milliequivalents per Kg). After $\frac{1}{2}$ hour the values of R for the viscera, especially the liver were 2 or more times as great as R for the plasma while R for muscle, skin, brain, and red cells was lower than plasma. The value of R for the carcass as a whole was somewhat less than the value for plasma. After 6 or more hours all tissues tend to approach the value for the carcass as a whole indicating complete exchange of radioactive K with normal K. After 10 hours about half of the red cell K, one third of the brain K and all of the muscle K has exchanged with radioactive K. Thus 30 minutes after injection 30 per cent of the marked K is in the viscera

which contain normally only about 16 per cent of the total K of the body. After 4-6 hours half of this amount or 15 per cent of the injected K has moved slowly from the viscera into the muscles and skin. Since the injected K enters the liver and other viscera 10-20 times as fast as it leaves it is probable that two different processes are involved. It is suggested that it enters the liver as an isotonic solution of K with some anion but leaves the liver and enters the muscles and red cells by the slower process of cation exchange.

Rapid impulses in large central fibres in fish. JAMES L. O'LEARY (by invitation) and H. T. GRAHAM. Washington University School of Medicine, St. Louis, Mo.

Certain nerve cells (Müller cells) of the nucleus motorius tegmenti of the catfish (*Ameiurus*) are characterized by their large size, paired paramedian distribution, and thick myelinated axons which, usually after decussation, pass into the ventromedian portion of the spinal cord (Bartelmez). These neurons are accessible to activation from the internal ear. From one pair (Mauthner cells) issue fibres of 35 microns average diameter. These may be identified at all cord levels among the 18 micron "Müller" fibres in the ventromedian area.

The medulla was stimulated through small needle electrodes in the paramedian area at the level of the facial lobe. Electrical responses were recorded from similar electrodes thrust into the ventromedian raphe of the spinal cord 30 to 100 mm. away from the point of stimulation. (The total cord length in the specimens used was about 110-150 mm.) The response usually appears as a group of overlapping waves which can be reduced in magnitude and number by decreasing the strength of stimulation; decreasing the strength of stimulation increases the magnitude and tends to produce a smooth wave. Waves of 8 microvolts following stimulation are readily distinguishable from the background activity of the cord, and waves of 500 to 1000 microvolts have been obtained with strong stimulation. The conduction rates and other properties of the responses resemble those of frog A fibre responses under similar conditions.

Electrical stimulation of the sacculi likewise produces a response in the cord, which appears later and tends to be more complex, protracted and of lower potential than the response to medullary stimulation. Under optimal experimental conditions, saccular stimulation also produces a propulsive movement. This suggests that the stimulated fibres participate in swimming reflexes, and therefore that they belong to the Müller-Mauthner system. The rapid rate of conduction of the responses induced by medullary stimulation, and the ventromedian situation from which they are recorded also suggests that they occur in the large fibres of this system.

Isoagglutinins in the blood plasma of dogs. WM. H. OLSON (introduced by H. Necheles). Departments of Gastro-Intestinal Research and Samuel Deutsch Serum Center, Michael Reese Hospital, Chicago, Ill.

A number of blood and plasma transfusions in dogs were followed by symptoms of shock. Compatibility tests showed that plasma often agglutinates red cells, while serum does so only occasionally. Compatibility tests for transfusions are performed by cross-matching donor's and recipient's serum and erythrocytes. This method, which has proved

adequate for demonstrating isoagglutinins in the human blood, has not yielded consistent results in dogs and reports are contradictory.

A total of 2614 tests were done on 85 dogs. The "army method" (glass slide) was used routinely and found satisfactory. The Landsteiner method was used at times to check the results; with the latter method great care must be taken to avoid hemolysis. Both plasma and serum from the same animal was used in the greater number of tests and were matched at the same time against the same set of erythrocytes. In the beginning of this work blood serum was obtained before the clot was completely formed, and an incidence of 15 per cent of positive agglutinations obtained in 308 tests. When the blood was placed in a refrigerator for several hours to allow for complete clotting, and the serum decanted without centrifuging, the number of positive agglutinations was reduced to $1\frac{2}{3}$ per cent in 624 tests. Blood plasma was used in 1682 tests with 743 positive agglutinations (44 per cent).

Our results show that dogs' plasma agglutinates dogs' erythrocytes in a high incidence of tests, i.e., 44 per cent, while dogs' serum agglutinates only in $1\frac{2}{3}$ per cent, provided that sufficient care has been taken to obtain complete clotting. This may explain contradictory reports from various laboratories. There appear to be at least 3 blood groups in dogs on the basis of our plasma agglutination studies. One group in which the cells are highly agglutinable and the plasma has no agglutinating power; another group in which the plasma is high in agglutinating power and the cells have none; and a third, possibly mixed group which after more extensive study may be further subdivided.

Circulation time in man at low temperatures. M. J. OPPENHEIMER and A. McCRAVEY (introduced by D. A. Collins). Departments of Physiology, and of Neurology and Neurosurgery, Temple University School of Medicine, Philadelphia, Pa.

It has been our opportunity to observe circulation time in humans at low temperatures during periods of refrigeration of patients carried out under the direction of Dr. Temple Fay, (Department of Neurosurgery) according to his new method designed to establish the effects of low temperatures upon embryonal cell growth (Smith and Fay, J.A.M.A. 113: 653, 1939).

Using the sodium cyanide method of Robb and Weiss (Am. Heart J. 8: 650, 1932-33) the following circulation times were recorded: a, arm vein (antecubital) to carotid and aortic chemoreceptors (carotid and aortic bodies); b, jugular vein to carotid and aortic chemoreceptors; c, femoral vein to carotid and aortic chemoreceptors. Skin temperatures were recorded by thermocouple, rectal temperatures measured by a resistance thermometer.

a. In all of 27 cases studied the interval between injection of sodium cyanide at an antecubital vein and arrival at the arterial chemoreceptors (= circulation time) was increased from an average of 17.2 seconds in those at normal body temperature, with and without anesthesia (controls) to 23.5 seconds in the same patients in hibernation. The extent of prolongation of circulation time showed no correlation with reductions in skin or rectal temperatures.

b. In all of 8 cases studied the interval between injection at the jugular and arrival at the arterial chemoreceptors was increased from an average of 11.9 seconds in the normal controls (with and without anesthesia) to

15.0 seconds in the same patients in the cold. There was no correlation between the extent of prolongation of circulation time and reductions in temperatures.

c. In all of eight cases studied the interval between injection into a femoral vein and arrival at the arterial chemoreceptors was increased from an average of 11.6 seconds in the normal controls (with and without anesthesia) to an average of 17.4 seconds in the same patients in the cold. In this group an apparent correlation existed between prolongation of circulation time and reduction in rectal temperature (approximately 5 per cent increase for each degree F fall in temperature).

*Oxidation-reduction potentials of carcinogenics and of growth stimulants.*¹

SEWARD E. OWEN. Cancer Research Unit, Veterans' Administration, Hines, Ill. (Read by title.)

Tissues contain the reducing substances ascorbic acid and glutathione. The latter decreases with age and this decrease seemingly parallels the animals' ability for tissue repair. The effectiveness of some growth stimulants depends upon the reducing condition in wounds, thus substances that stimulate tissue growth might be suspected of being able to increase the reducing power of buffered solutions. Since the carcinogenics stimulate tissue growth a comparison of these with known tissue growth stimulants is of interest from the oxidation-reduction angle.

Glass electrode pH determinations and gold over platinum Eh values were determined on buffer controls, cysteine, glutathione, methyl cholan-threne, allantoin, dibenzanthracene, desiccated larva suspensions, benzpyrene, indole-3-acetic acid, triphenyl benzene and cystine. At constant pH lowered Eh and rH readings occurred with all but were the greatest with the sulphydryl chemicals. The lowering of the rH values and Eh voltages for the carcinogenics occurred in either acid or alkaline phosphate buffer. The carcinogenics did not affect the chemical tests for free sulphydryl or the sulphydryl content of proteins (albumin and edestin) in vitro. Urea, suspensions of desiccated larva and allantoin in contact with these proteins in solution released sulphydryl which was detectable chemically or by electrometric means. The mechanism for growth stimulation by the carcinogenics could not thus be shown to be concerned directly with sulphydryl in vitro although both the carcinogenics and known growth stimulants increased the reducing power of buffered solutions. It is of interest to point out that the rH and Eh values of tumor tissue and of embryonic tissue are lower than those for muscle which may be thought attributable to the special cellular metabolism common to these tissues. Sufficient materials being present for constructive metabolism it is believed that an increase of reducing power is associated with increased healing ability of wounds. Evidence is not at hand to indicate that increasing values of rH (denoting increased oxidizing power) might be associated with delayed wound healing.

Angiotonin-activator and renin and angiotonin inhibitor. IRVINE H. PAGE and O. M. HELMER (by invitation). Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind.

¹ Published with the permission of the Medical Director of the Veterans' Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

Angiotonin, the pressor substance resulting from interaction of renin and renin-activator, causes tachyphylaxis to develop slowly when injected into animals, in marked contrast to renin. But establishment of renin tachyphylaxis inhibits the action of angiotonin as well.

Angiotonin requires an activator to exert its pressor action in perfused isolated organs. This was prepared from ox-serum or red blood cells by fractional precipitation with potassium phosphate at pH 6.5. It differs from renin-activator.

When angiotonin tachyphylaxis is developed in an animal, the angiotonin-activator is lost.

Bilateral nephrectomy greatly increases the sensitivity of animals to both angiotonin and renin. When an isolated organ is perfused with blood from such animals the pressor response elicited by injecting angiotonin or renin is much enhanced. This, along with other evidence, suggests that the increased sensitivity is due to loss of an inhibitor normally supplied by the kidneys and possibly other tissues. Extracts of kidneys lower the blood pressures of hypertensive dogs and rats.

X-ray diffraction studies of nerve lipides. KENNETH J. PALMER (by invitation), RICHARD S. BEAR (by invitation) and FRANCIS O. SCHMITT. Department of Zoology, Washington University, St. Louis, Mo.

To facilitate the analysis of the molecular organization of the nerve axon sheath, the plasma membrane, and other lipide-protein structures, x-ray diffraction patterns were obtained from lecithin, cephalin, sphingomyelin, phrenosin, kersasin, and cholesterol, both as single components and in multiple-component mixtures and both in the dry condition and when wetted with varying amounts of water.

These substances occur as double molecular layers, which when in the dry condition give characteristic long spacings. These are approximately 64 AU. for spingomyelin, phrenosin, and kersasin, 43 AU. for lecithin and cephalin and 33.9 AU. for cholesterol. From dry multiple-component systems as well as from dry nerve, these three spacings are obtained, the intensity of each diffraction depending upon the amount of each substance present. When wet with water a coacervate system is formed, the mixed lipides associating in double layers, give a single long spacing, the value of which may be as high as 120 AU. depending upon the amount of water separating the double layers. When the water content is about equal to that in the myelin sheath the long spacing is 70-80 AU. This shows that the spacing of 170-180 AU. obtained from the fresh myelin sheath is not due to the mixed lipides alone, but that protein must contribute to the fundamental period. From the diffraction evidence and from analytical figures for the amount of protein and lipide in the myelin sheath the probable structure of the smectic fluid crystalline layers in the radial direction has been deduced. In this structure double layers of mixed lipides alternate with monolayers of proteins, the lipide double layers being bonded to one side of the protein monolayers by their hydrophilic groups and to the other side by their hydrophobic groups. This structure differs from that of cephalin-protein complexes (studied in collaboration with Dr. E. Chargaff) in that, although the latter contain monolayers of protein intercalated between double layers of cephalin, the small identity period indicates that the protein monolayers do not have predominantly hydrophilic and hydrophobic surfaces.

Iron and orthophenanthroline as accelerators of brain tissue oxygen consumption. FRIEDA PANIMON (by invitation), M. K. HORWITT (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Chicago, and Biochemical Research Laboratory, Elgin State Hospital, Elgin, Ill.

Ferrous and ferric salts, added to brain brei (rat) in vitro, increase its oxygen consumption. One per cent FeCl_3 can increase by over 9 times the oxygen used by a 1 to 40 suspension of brain in Ringer solution, and lower ferric concentrations have decreasing effects. FeCl_2 and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ give a five-fold increase at corresponding concentrations. Ortho or pyrophosphate abolishes the ferric effect, acetate stops or much decreases the ferrous action. Though succinate, lactate, glucose, and pyruvate increase the basic oxygen consumption they do not augment the iron action; nor does urethane, which inhibits the brei, or malonate, decrease the iron action. Ferric ion was regularly tested in acetate buffer, ferrous in phosphate, in which it is gradually oxidized.

The systems oxidized with the aid of iron are thermostable, are not extracted by acetone or trichloroacetic acid, can be centrifuged from suspension, and are not used up in the course of autoxidation. They are not, however, non-specific. No single protein or lipid tested showed an iron effect; and kidney and spleen brei, unlike brain and liver, are actually inhibited by ferric, mildly enhanced by ferrous ion.

While attempting to form various iron complexes, orthophenanthroline was used. This dye, and its ferrous complex, proved extremely effective in increasing the oxygen usage of brain brei. The optimal concentration of dye, less than 0.2 mM, tripled oxygen uptake even two hours after addition and a 10^{-5} molar concentration doubled it. Stronger concentrations gave less acceleration or an inhibition. Further details of this striking dye catalysis on brain oxidations will be reported.

Vasoconstrictor nerve and oxygen consumption in the isolated perfused hindlimb muscles of the dog. J. R. PAPPENHEIMER (introduced by H. C. Bazett). Department of Pharmacology, University College, London, and the Laboratory of Physiology, University of Pennsylvania, Philadelphia.

Stimulation of the sciatic nerve in the isolated perfused curarized hindlimb preparation of the dog causes a reduction of both blood flow and arterio-venous difference in oxygen content. The oxygen-consumption is therefore apparently reduced and more than in proportion to the blood flow (J. Pappenheimer, Demonstration to the Brit. Physiol. Soc., March 1939).

The changes in blood flow and oxygen saturation are similar to those which Rein and Schneider (Pflüger's Arch. **239**: 464, 1937) have described as occurring in the intact hindlimb of the dog during vasoconstriction elicited reflexly from the carotid sinus. Rein and Schneider have concluded that the sympathetic nerves exert a direct action in lowering the oxygen-consumption and heat production of muscle.

In the experiments to be described in this paper the blood flow and oxygen-saturation in the isolated preparation were continuously recorded by the methods of Kramer and Winton (J. Physiol. **96**: 87, 1939). Evidence has been obtained for an alternative explanation of the phenomenon which makes unnecessary the conclusion of Rein and Schneider that the

sympathetic nerves have a direct action on the metabolism of muscle. The evidence suggests that the action of the vasoconstrictor nerves is to clamp off the circulation completely in parts of the tissue and to divert blood through regions in which both the oxygen-consumption and the surface available for heat loss are low. The evidence has come from the following lines of inquiry.

1. Comparison of the effects of changes in blood flow caused by changing the arterial pressure or by the action of adrenaline with those caused by stimulation of the nerve.

2. The quantitative comparison of the apparent reduction in oxygen-consumption occurring during the stimulation with the apparent increase which follows release of the stimulation.

3. A study of the effects of varying the frequency of stimulation.

4. The action of ergotoxine.

5. The arteriovenous temperature difference.

Electrotonus and accommodation in the excised and circulated frog's sciatic nerve. HORACE O. PARRACK (introduced by Kenneth S. Cole). Department of Physiology, Columbia University Medical School, New York City. (Read by title.)

The excitability of the alpha fibers in the frog's sciatic nerve during the passage of a subrheobasic constant current has been measured by determining the strength of a short test shock necessary to evoke half maximal alpha spike potentials. As found by other investigators, the alteration of excitability in excised nerves is maximum at about one millisecond after the application of the constant current and generally falls to a steady value different from zero. The decrease and the final value of excitability are measures of the amplitude of accommodation and electrotonus respectively.

Excised nerves not exposed to Ringer's solution show both accommodation and electrotonus. Ringer's solution containing excess Ca^{++} reduces the amplitude of accommodation and may make it zero or negative, but excess K^{+} increases the amplitude of accommodation and shortens the time to half accommodation.

Nerves with intact circulation in spinal animals show a negligible accommodation up to one second after application for currents 50 to 90 per cent of rheobasic. After the circulation is blocked or during anesthesia, the amplitude of accommodation becomes comparable to that of excised preparations.

In all preparations the effects at anode and cathode are not mirror images of each other. When present, accommodation is always less at the anode than at the cathode, and negative accommodation in circulated preparations and excised preparations subjected to excess Ca^{++} is more marked at the anode.

If other experiments also demonstrate that accommodation is negligible under nearly normal physiological conditions, it should be considered as an abnormal alteration of electrotonus, excitability or threshold which is characterized by an amplitude as well as a time constant. The two factor theories of excitation allow this viewpoint if the minimal gradient and allied phenomena may be classified as abnormal.

Accumulation of chloride in the ileum under conditions suitable for active chloride absorption. H. C. PETERS (introduced by Lathan A. Crandall,

Jr.). Department of Physiology, University of Tennessee, Memphis. Ingraham, Peters, and Visscher (J. Phys. Chem. **42**: 141, 1938) proposed the theory that the absorption of water "washes" chloride out of the intestinal contents without changing its concentration while a fluid practically free of chloride moves into the intestinal lumen.

The known facts of secretion and diffusion of chloride into initially chloride-free solutions in the intestine have been recognized by Peters and Visscher (J. Cell. and Comp. Physiol. **13**: 51, 1939), who state that these factors are practically negligible at high rates of absorption.

In order to obtain more definite information under the conditions of these experiments, accumulation of chloride in a solution containing $\frac{1}{2}$ isotonic sodium sulfate and $\frac{1}{2}$ isotonic sodium nitrate was studied in ileal loops of anesthetized dogs. The average concentration found at the end of a ten minute period was 0.05 per cent NaCl. The average net water absorption in this period was 6 per cent. These experiments seem to indicate that chloride movement into the intestine is not negligible at slow or even moderate rates of absorption.

The following more general equation has been derived:

$$\log \frac{(K + R_i)C - C_oS - KC_b}{(K + R_i)C_o - C_oS - KC_b} = \frac{K + R_i}{D} \log \frac{V}{V_o}$$

This equation takes into account osmosis as well as diffusion and secretion of chloride, but can be applied only when the rates of water flow are approximately constant.

The attenuation of parathyroprival tetany by double vagotomy above the diaphragm. CARL PFEIFFER, CHARLES ROBY and SIDNEY SMITH (introduced by Arno B. Luckhardt). Department of Pharmacology, University of Chicago, Chicago, Ill.

On finding that the serum Ca and K rose after excitement in the dog and that this rise could be minimized by bilateral vagal section, it was thought that this operation might attenuate parathyroprival tetany. A decrease in Ca excretion after excitement was noted if the vagi had been sectioned 2-8 weeks previously. Six of 7 totally thyroidectomized control dogs died of acute tetany with an average survival time of 7.7 days. Five of 6 dogs which had a total thyroidectomy and double vagotomy during the same anesthesia died of fulminating, acute tetany on the 3rd or 4th day. In a series of 12 dogs with vagi sectioned above the diaphragm 2-8 weeks before total thyroidectomy, 5 died "depressive deaths" without acute tetany. The serum Ca fell to 3.0 mgm. per cent in some of these dogs without precipitating acute tetany.

Diabetes insipidus in the hypophysectomized rat. R. A. PHILLIPS and H. GILDER (by invitation). Department of Physiology, Cornell University Medical College, New York City.

The fluid exchange has been studied on normal and hypophysectomized rats, and rats with section of the stalk of the pituitary. Food (dry) and fluid intake are based on 3 day averages and were extended for over 2 months after operation. Study of serial sections of the decalcified brain bases in the pituitary region verified the supposed operative procedures. The specific gravity of the fresh urine, obtained by abdominal pressure,

was determined by the Hammerschlag Method. The specific gravity of the urine of the normal rats was always above 1.050.

Hypophysectomy results in a definite polydipsia and polyuria which is not always obvious unless the fluid exchange is related to body weight. This diabetes insipidus is not maximal presumably due to the varying amounts of normal appearing pituitary stalk still remaining. The substitution of 0.5 per cent and 1.0 per cent NaCl for H₂O resulted in an increased polydipsia and polyuria. However, in no instance did the specific gravity of the urine of the hypophysectomized rats exceed 1.010 even when 1.0 per cent NaCl was substituted for H₂O.

*Oestrogen excretion of certain cancerous and non-cancerous individuals.*¹

GREGORY PINCUS and MARK GRAUBARD (by invitation). Physiological Laboratories, Clark University, Worcester, Mass.

Separation into oestrone and oestriol fractions was made of a series of urines from non-cancerous, normally cyclic, post-menopausal, ovariectomized, and hysterectomized women before and after the injection of 1 oestrone, and 2 oestrone plus progesterone. Bioassays of these fractions show only slight activity in the oestriol fraction in post-menopausal, ovariectomized and hysterectomized women and a low total oestrogen output compared to normally cyclic women. After the injection of oestrone increased activity is observed in both fractions in normally cyclic women but not in the oestriol fraction of those presumably lacking functional uteri. After oestrone plus progesterone is administered the normally cyclic women show a higher activity in the oestriol fraction indicating conversion of oestrone to oestriol and all women show a heightened total oestrogen output.

In a series of comparable women having cancer of the uterus and cervix there appears to be before hormone administration, on the average, a larger total oestrogen output with the activity distributed more or less equally between the oestrone and oestriol fractions. Oestrone administration leads to a somewhat increased total oestrogen output but the simultaneous administration of oestrone and progesterone causes no appreciable enhancement of oestrogen output and gives no indication of oestrone to oestriol conversion.

These data may be interpreted as demonstrating either an abnormal destruction of administered oestrogen in the cancerous women or the conversion of the hormone to an atypical oestrogenic material. Evidence for the latter hypothesis will be presented.

Action of atmospheric pressure on latent thermolysis. J. PI-SUNER (introduced by J. F. Fulton). La Casa de Espana en México and Department of Physiology, Escuela Nacional de Medicina, Mexico.

The importance of loss of heat by aqueous evaporation is a fundamental idea of thermic regulation. Our experiments were made with the Mayer technique with rabbits placed comfortably in special tunnels restricting movement but not aqueous vaporization. We used postabsorptive 1.8 to 2.0 kgm. animals accustomed to experimentation in 2 hour runs. The average loss of weight under these conditions observed in Paris and Barcelona, is from 0.07 to 0.10 gram per kgm. per hour, but with important individual variations. The following table gives results of typical experiments in Mexico:

¹ Aided by a grant from the Ittleson Foundation.

LOSS OF WEIGHT	ATMOSPHERIC PRESSURE	TEMPERATURE IN °C.			
		Initial		Final	
		D.T.	H.T.	D.T.	H.T.
<i>grams per kgm. per hour</i>	<i>mm. Hg</i>				
0.094	581.9	19.0	16.0	19.1	16.1
0.080	587.7	20.0	16.0	19.6	15.5
0.136	584.7	21.0	17.2	19.9	16.2
0.081	555.2	20.1	16.3	19.1	16.1
0.050	584.5	18.7	17.0	18.0	16.2
0.084	565.7	16.6	15.2	16.0	14.6
0.107	587.0	15.9	15.0	15.3	14.2

Apart from individual variations, water loss is thus within normal limits. A new series of observations was now made with animals in exactly the same conditions in a chamber with atmospheric pressure 200 mm. higher and therefore practically at sea level pressure, with renovation of air but without draft which might modify the vaporization. The last four animals of the preceding table were studied under these conditions. The results are here:

0.082	578.6	19.6	17.1	19.8	17.2
0.060	584.5	18.7	17.0	18.0	16.2
0.085	555.0	20.1	16.3	19.1	16.1
0.115	584.7	21.0	17.2	19.9	16.2

The vaporization of water which assures the thermolysis in latent form is made, as has been seen, with absolute indifference to the atmospheric pressure. The relation with the surrounding vapor pressure of water seems to be narrower; observations in this field are being continued.

The rôle of the hypothalamus in cardiovascular regulation. R. F. PITTS, M. G. LARRABEE (by invitation) and D. W. BRONK. Johnson Foundation, University of Pennsylvania, Philadelphia.

In order to evaluate the rôle of the hypothalamus in cardiovascular regulation we have compared the character of the spontaneous activity in peripheral sympathetic nerves, as well as its reflex modification, before and after extirpation of the hypothalamus. In addition we have studied the activity induced in these nerves by stimulation of the hypothalamus. In these latter experiments stimulating electrodes were oriented in the supra-optic and tuberal portions of the hypothalamus by means of the Horsley-Clark stereotaxic instrument, and brief repetitive condenser discharges delivered at controlled intensities and frequencies.

The characteristic grouping of spontaneous activity in the various fibers of the sympathetic nerve trunks and their reflex inhibition and excitation were in no wise altered by extirpation of the hypothalamus.

Stimulation of the hypothalamus at frequencies above 10 per second produced an increase in sympathetic activity in the inferior cardiac and cervical sympathetic nerves, and after a brief latency a rise in blood pressure. Frequencies from 1 to 10 per second often produced an inhibition of sympathetic activity and a fall in blood pressure. Records from few fiber preparations indicated that the individual units discharged more

frequently and that more units were brought into activity as either stimulus frequency or intensity were increased.

It has been possible to record from single fibers of the cervical sympathetic activated from the hypothalamus. Frequency of discharge was greatest at the beginning of stimulation, but after a period of a second or so a steady state was attained and the unit discharged rhythmically, the frequency of discharge being directly related to the intensity and frequency of hypothalamic stimulation.

There is, following stimulation of the hypothalamus, an abrupt inhibition of all spontaneous sympathetic activity and a reduced excitability of the lower sympathetic centers to either reflex excitation from afferent nerves or to hypothalamic stimulation.

These experiments show how the chemical and reflex control of sympathetic centers is modified by varying degrees of hypothalamic activity.

Distribution of vitamin A in the rat as studied by fluorescence microscopy.

HANS POPPER and RUVEN GREENBERG (introduced by F. T. Jung). Cook County Graduate School of Medicine, and the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Vitamin A was examined in organs of rats by means of fluorescence microscopy and microphotography (Popper) and the characteristic green fluorescence fading upon irradiation with ultraviolet light was studied. The results were usually checked by chemical assay of the liver for vitamin A (Carr-Price reaction). In vitamin A deficient rats the green fluorescence was not seen except in the retina. There it remained even in advanced deficiency.

In normal rats various amounts of the characteristic fluorescence were found in the liver in Kupffer and epithelial cells, in the latter in lipid droplets or diffusely in the cytoplasm. It was seen in the adrenal cortex in small droplets. It was seen in the corpus luteum and the interstitial cells of the ovary. (The testes were free.) The fat cells contain moderate, the retina rather high amounts. Traces of the fluorescence may be found in the interstitium of the renal cortex and papilla, in the alveolar septa of the lungs, and the intermediary part of the pituitary. If vitamin A was fed the fluorescence was visible within the lumen of the stomach and within the lumen and wall of the small intestine. It could be traced through the epithelial lining and lymphatics in the villi to larger lymphatics through to the subserous layer. The peak of absorption occurs in the jejunum. The fluorescence was not seen in epithelium except for the sites mentioned.

In hypervitaminotic animals the tissue storage was increased; this was especially evident in the Kupffer cells. Furthermore, it was visible in the pleura, epicardium, peritoneum, and meninges.

Four hours after feeding of 4,000 I.U. of vitamin A to deficient rats the vitamin fluorescence became simultaneously visible in Kupffer and adjacent epithelial cells of the liver and in the adrenal and ovary in minute amounts. With intraperitoneal and subcutaneous administration more time was required. With oral administration of beta-carotene the fluorescence appeared in Kupffer cells and in minute amounts in epithelial cells of liver and adrenal only after 24 hours.

Pancreatic reflux and bile tract. H. L. POPPER (by invitation) and H. NECHELES. Department of Gastro-Intestinal Research, Michael Reese Hospital, and Department of Physiology, University of Chicago, Chicago, Ill.

The author has demonstrated previously the occurrence of diastatic and tryptic enzymes in human gallbladder bile and has demonstrated that these pancreatic enzymes pass from the pancreatic into the common bile duct, provided that both ducts form a common channel. The author has shown that the mixture of pancreatic secretion and bile may penetrate the intact wall of the gallbladder and thus produce bile peritonitis.

The present report deals with the investigation of this phenomenon on another hollow viscus, the small intestine. In anesthetized dogs the duodenum was ligated below the pylorus and distally to the entrance of the main pancreatic duct. Secretion of the pancreas and of liver bile was stimulated by subcutaneous injections of mecholyl, eserine and decholin. The loop appeared distended after two or three hours. The experiment was continued for five to seven hours at the end of which the loop was examined. A bloody peritoneal fluid was present and was examined likewise. The walls of the duodenum were found to be both grossly and microscopically normal, but the exudate in the peritoneal cavity contained a high concentration of pancreatic enzymes. The pancreas was found to be normal.

In further experiments a loop of jejunum was tied off, a cannula inserted and the loop distended by injection of a mixture of dogs' bile and a 1 per cent solution of a 1/200 commercial trypsin; after 4 hours the experiment was terminated and the jejunal loop examined. No change was observed grossly or microscopically. It is concluded that the mixture of pancreatic juice and bile, per se, does not damage the gallbladder or the intestine, and that changes observed under such conditions are due to concomitant pathological lesions of these organs, or to the permeability of the normal organs to a mixture of bile and pancreatic juice.

The effect on the spinal reflex threshold of intravenous injections of potassium chloride. E. L. PORTER, EDWARD VOGEL (by invitation) and J. E. ROBERTSON (by invitation). Department of Physiology, Medical School of the University of Texas, Galveston.

In the spinal cat intravenous injections of 0.9 per cent potassium chloride result in a lowering of flexion reflex threshold which occurs both in the normal animal and in the animal with reflex threshold raised by the injection of a barbiturate. This may or may not be due to the influence of potassium on synaptic conduction within the cord. It may be due to pain. A drop or two of boiling water placed on the skin of the hind leg will cause a lowering of the flexion reflex threshold which may be as pronounced and persist as long as the effect of the injections of potassium chloride. Moore (Am. J. Physiol. **110**: 191, 1934) has shown that pain follows the injection of potassium chloride into the artery of a cat under a barbiturate. Our calculations, however, indicate that our results are obtained at a lower concentration of KCl in the blood than Moore's minimum for causing pain. The weight of the evidence, therefore, supports the view that the potassium in our experiments is acting on the synapses of the cord.

*Gastric evacuation: Correlation of antral and bulbar pressures with fluoroscopic observations.*¹ J. P. QUIGLEY, J. M. WERLE (by invitation) and DANIEL BRODY (by invitation). Department of Physiology, Western Reserve University Medical School, Cleveland, O.

Fluoroscopic studies and optical recordings of intralumen pressure were made from the antrum and bulb of trained dogs while evacuating 100 cc. of strained corn meal meat mush and 30 grams BaSO₄ from the stomach.

A contraction wave begins at the incisura and four seconds later pyloric evacuation and bulbar filling begin without significant antral or bulbar pressure changes. Two or three seconds later antral pressure begins to rise and at, or before, its maximum, evacuation ceases and bulbar pressure rises sharply.

Basal antral pressures are 4 to 7 cm. of water; bulbar pressures 0.5 to 3 cm. Pressure waves in the antrum occur 4 to 5 times per minute with maximal pressures of 25 to 30 cm. and persist 5 to 7 seconds. In the bulb, waves of about the same frequency and magnitude are related to the antral waves.

Bulbar emptying typically begins one second after completion of bulbar filling but it occasionally begins during the filling process. Bulbar evacuation requires 1 to 4 seconds.

Following the ingestion of one liter of corn meal mush, observations made at 15 minute intervals show a continuous repetition of the pressure cycle for three hours; subsequently, the pressure waves may be periodically depressed to maxima of 8 cm. for ten minute intervals.

These observations ascribe gastric evacuation to the moderate basal pressure gradient from antral to bulbar region. The antral peristaltic wave which induces evacuation does not significantly alter these basal pressures. During the period of maximum ejection, resistance to propulsion evidently is slight; the sphincter and bulb offer little resistance. A significant rise in antral pressure can occur when a contraction wave is associated with a resistance to propulsion. This situation develops shortly prior to the end of antral contraction. Apparently at this period the sphincter is closed, and duodenal regurgitation as well as gastric evacuation is prevented, while antral and bulbar pressures can rise significantly.

An effect of the extractive fraction of meat in promoting fatty infiltration of the livers of depancreatized and pancreatic-duct-ligated dogs. ELAINE P. RALLI and SAUL H. RUBIN (by invitation). Laboratories of the Department of Medicine, New York University College of Medicine, New York City.

The occurrence of fatty livers has repeatedly been reported (Best, Chalkoff, Ralli, etc.) in depancreatized or pancreatic duct-ligated dogs fed whole meat. When meat powder (Valentine), from which the extractives have been removed, was substituted for whole meat, little or no fatty infiltration of the liver occurred. The diet contained meat powder, cracker meal, skimmed milk powder, brewers' yeast, salts, cod liver oil and corn oil. In 6 depancreatized and 3 duct-ligated dogs fed this diet, for periods varying from 6.5 to 19 weeks (average 12.5 weeks), the fatty acid content of the entire liver ranged from 3.12 to 10.5 per cent (average 6.2 per cent).

Another group of depancreatized and duct-ligated dogs was fed this diet

¹ This investigation was aided by a grant from the American Medical Association.

plus 15 cc. of meat juice (Valentine) for 5 to 9 weeks. The livers of these dogs contained 18.3 to 19.8 per cent total fatty acids. It is therefore concluded that the extractive fraction of meat contains a substance which increases fat deposition in the livers of these animals.

Graded amounts of the meat juice were fed to rats on a high-fat, low-protein diet with a slight *lipotropic* effect. Whole meat and meat powder had a manifest lipotropic effect. These discrepant observations show that the alimentary fatty liver of the rat and that of the depancreatized dog do not necessarily respond to dietary supplements in the same way.

Respiration and cell composition of frog brain regions. JANE REHM (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Chicago, Ill.

Bits of frog brain (1 to 3 mgm.) were obtained under a dissecting microscope from desired regions; their oxygen consumption followed in a capillary respirometer, beginning 5 to 15 minutes after decapitation, for an hour; and they were then macerated and the number and size of the contained cell nuclei were determined by a hemocytometer technique. Controls on blood and fresh brain and duplicates on bilateral structures proved that there is no regular loss of nuclei and that counts check, on the average, better than 15 per cent.

Nuclei of different cell types fall into rather well limited size groups: those of red blood cells cluster around 5 by 10 μ ; endothelium (from blood vessels), 15 to 25 by 5; neurones (and glia) of the cerebellum (plus commissure), 10 by 10; neurones of the anterior olfactory nuclei, 10 by 15; neurones of the hippocampus, 15 by 20. Total cells per cubic millimeter for these neural structures, in the above order, are 200,000, 135,000, and 70,000; and, in each, nerve cells constitute 75 per cent of the total number. From the number of blood and endothelial cells, the volume per cent of blood and the relative capillary length can be estimated.

Respiration (last half-hour of run) per million nerve cells, averages 3, 5 and 10 cu. mm. per hour for these same three brain structures, cerebellum, olfactory nuclei, and hippocampus; per gm. fresh weight, it averages 350, 350 and 500. Other values, on a weight basis, are: hypothalamus, 400; primordium palii, 600. Average volume of cell nuclei, respiration per milligram, and respiration per cell vary together and inversely to cells per milligram.

Activity of the simplest reflex pathways in the spinal cord. BIRDSEY RENSCHAW (introduced by Herbert S. Gasser). Laboratories of The Rockefeller Institute for Medical Research, New York City.

A shock exciting $\frac{1}{4}$ to $\frac{1}{2}$ of the alpha fibers in a lumbar or sacral dorsal root of the decerebrated cat typically produces in the corresponding homolateral ventral root a reflex discharge with a central delay of 0.65 to 1.0 msec. Conditioning the cord with a dorsal root volley decreases the central reflex time slightly, but no procedures have reduced it below 0.5 msec. Ventral root discharges resulting from direct electrical stimulation of the central gray matter, after the technique introduced by Lorente de N6, demonstrate that the synaptic delays at the motoneurons vary over a similar range of values. Consequently central reflex times of 0.5 to 1.0 msec. represent activity in arcs of two neurons (one synaptic relay). It

is difficult to test whether longer synaptic delays sometimes occur, for central reflex times as short as about 1.0 msec. may conceivably pertain to activity in arcs of three neurons.

The earliest motoneuron discharges are followed by others at all central reflex times up to several milliseconds,—a necessary result if the synaptic delays at interneurons as well as at motoneurons range between the limits 0.5 and 1.0 msec. The synapse times show modes, and large groups of motoneurons are typically discharged at specific central reflex times; nevertheless, variation of the delays is sufficient to preclude calculation from the central latency of the exact number of synaptic relays involved in the production of all except the earliest discharges.

Activity in two-neuron pathways is conditioned by a contralateral volley. Although the latter fires few motoneurons, it facilitates others and the two-neuron discharges are augmented during the small contralaterally evoked discharge ($15 \pm$ msec.). Facilitation is followed by a period of inhibition in which the deficit in the response of two-neuron pathways is approximately equivalent to the entire crossed discharge. Consequently, if the motoneurons discharged by the homolateral volley are the same as those fired by the contralateral stimulus, the inhibition may be assigned to subnormality of motoneurons. During the same period the tonic background of activity in the ventral horn is diminished; withdrawal of its facilitating effect may also contribute to the deficit.

Endocrine and behavior effects of vitamin B deficiency studied in rats by the self-selection method. CURT P. RICHTER and CLARENCE D. HAWKES (by invitation). Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md.

Rats given free access to sucrose, olive oil, casein (autoclaved and purified), 5 minerals, cod liver oil, and dried baker's yeast in separate containers made selections which resulted in normal growth and reproduction. After the removal of yeast, which completely eliminated vitamin B from the diet, rats lost weight and showed dioestrous vaginal smears and certain appetite changes, consisting of an aversion for carbohydrate and protein and a craving for fat. They died after an average of 65 days on such a diet.

Animals autopsied after 40 days showed marked atrophic changes in the endocrine glands and related digestive glands and organs of the female reproductive tract. Crystalline preparations of B₁, riboflavin, nicotinic acid, and B₆, offered separately or in various combinations, had a beneficial effect; and almost complete replacement was obtained in animals receiving all the components.

Previously we have shown that thiamin chloride alone restores the carbohydrate appetite to normal and that combination of this vitamin with riboflavin and nicotinic acid partially restored protein appetite. In these experiments the addition of crystalline B₆ to the other three components resulted in the restoration of the protein appetite to the normal level and cessation of the craving for fat. Thus, the different components of the B complex seem to have a synergistic action. The failure of rats on a choice of all four components to grow at the same rate as the controls indicates that some other factor present in yeast must still be lacking in the choice offered them.

Further observations on the calorogenic action of fat in pancreatic diabetes.

GORDON C. RING. Department of Physiology, The Ohio State University, Columbus.

Several years ago, it was observed that the ingestion of oleic acid by partially depancreatized cats produced a far greater calorogenic effect than a similar ingestion by normal animals. It has not been possible to produce as severe diabetes in rats as in cats. Consequently, it was important to see how the fat metabolism of rats would be affected by pancreatectomy. Using the operative procedure described by Shapiro and Pineus, pancreatic tissue has been extirpated as completely as possible from month-old rats. It is believed that the removal was more complete than in the experiments performed on cats where at least 5 per cent of the pancreas was intentionally left behind. When the operated rats had reached a weight of 150 gm. or more, the calorogenic effect of ingested oleic acid was found to be within the range obtained for normal animals. However, if these animals were given a diet containing one gram of olive oil per day, the specific dynamic action of oleic acid increased to levels similar to those observed in diabetic cats.

Electrical axis and monocardigrams in hypertensive individuals. JANE

SANDS ROBB and ROBERT C. ROBB (by invitation). Department of Pharmacology, Syracuse University, Syracuse, N. Y.

The electrical axis throughout the cycle has been calculated from simultaneous electrocardiograms of 100 hypertensive individuals, patients of Dr. Bertram Levinson at the Syracuse Free Dispensary. Monocardigrams have also been calculated by the method of Mann. A comparison of electrocardiograms, electric axis, monocardigrams, and various clinical data has been made. Several types of graph appear in the series which can be analyzed to limit the spread of the so-called "normal" axis as well as to indicate what axis definitely indicates Left deviation.

Studies of temperature regulation in white men and negroes. SID ROBINSON

(by invitation), D. B. DILL, F. G. HALL and J. W. WILSON (by invitation). Indiana University, Bloomington, Harvard Fatigue Laboratory, Cambridge, Mass. and Duke University, Durham, N. C.

Twenty negro sharecroppers and seven white sharecroppers were studied during a two-hour walk on a motor driven treadmill. The work was severe enough to elevate metabolism to about eight times its basal level in the average man. Both groups were thoroughly acclimatized by work in the cotton fields. The experiments were carried out in Mississippi in mid-summer in a room where the air was still and the temperature averaged 30.2°C. with an average humidity of 78.4 per cent. Under these conditions the rate of sweating was in excess of evaporation in all men, the skin being thoroughly wet soon after work began. The negroes were able to attain a balance between heat production and heat loss after 30 minutes of work and continue the work to the end with an average rectal temperature of 38.2°C. The rectal temperatures in the white men continued to rise for 105 minutes and they finished with an average of 38.6°C. The negroes were slightly more efficient in the walk, requiring an average per minute of 24.9 cc. of O₂ per kilogram of body weight in the first fifteen

minutes of work and 25.6 cc. at the end as compared with 25.9 and 27.3 in the white men. The heart rates of the negroes reached an average of 152 as compared with 173 in the white men.

Two negroes, one a house servant and the other a drug store clerk, attempted the same walk. Neither was able to reach a steady state and their rectal temperatures continued to rise in a straight line until they were forced to stop—one with a temperature of 39.2°C. after 52 minutes of walking, the other with a temperature of 40.1°C. after 92 minutes. These men were less efficient and their heart rates reached higher levels than the negro field workers.

Amino-acids and hemoglobin formation. F. S. ROBSCHT-ROBBINS.

Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

The effect of various amino-acids (natural forms and optical isomers) on hemoglobin formation in an anemia due to blood withdrawal in dogs has been investigated. These dogs are kept continuously anemic for a period of years at a hemoglobin level of about one-third of their normal value. These animals are well standardized and their response in hemoglobin production to various factors is well established. The pure crystalline amino-acids are added to a basal ration of a salmon bread mixture. Among those studied are proline, cystine, glycine, glutamic acid, tryptophane, tyrosine, leucine, isoleucine, lysine, methionine and arginine. The results obtained indicate a varying degree of potency for hemoglobin production under these experimental conditions.

*Direct arterial pressure in the unanesthetized rabbit.*¹ S. ROBBARD (introduced by L. N. Katz). Cardiovascular Dept., Michael Reese Hospital, Chicago, Ill. (Read by title.)

In the past the blood pressure in the rabbit has been measured indirectly without anesthesia by various methods and directly by means of arterial cannulation after anesthetization. The need for direct arterial pressure determinations on the normal unanesthetized rabbit led us to develop the following technique. Rabbits can be quieted without the use of drugs by placing the animal on its back and stretching the forelegs cephalad and the hind legs caudad. After 10 to 15 seconds in this position the legs can be released and the animal will make no attempt to right itself. The femoral artery can then be punctured with a needle to which a mercury or a Hamilton manometer is attached.

We have found that the blood pressure of the normal unanesthetized rabbit obtained in this way with a Hamilton manometer was 110/80 mm. Hg with a range in 32 animals of from 130 to 95 mm. Hg in the systolic and 90 to 60 mm. Hg in the diastolic pressure; pulse pressure—25 to 30 mm. Hg.

*Elimination of the chemical mediator of renal hypertension by renal metabolism.*² S. ROBBARD (introduced by L. N. Katz). Cardiovascular Dept., Michael Reese Hospital, Chicago, Ill.

We have previously shown that the effect of the chemical mediator of

¹ Aided by a grant from the Dazian Foundation.

² Aided by the A. D. Nast Fund for Cardiac Research, and a grant from the Dazian Foundation.

renal hypertension can be dissipated at a rapid rate only in the presence of kidney tissue (Rodbard and Katz, *Am. J. Med. Sci.* **198**: 602, 1939). When a normal kidney remains in the body, the diastolic arterial pressure returns from hypertensive levels to normal within six hours after removal of the ischemic kidney responsible for the increased pressure. However, after total nephrectomy, the blood pressure falls more slowly and reaches the normal level on the average in twenty-five hours.

We have undertaken to determine whether the excretory or metabolic functions of the kidney are responsible for the elimination of the renal pressor principle. Blood pressures were determined by means of the Hamilton manometer on unanesthetized trained dogs. Hypertension was produced by means of unilateral renal ischemia, using the Goldblatt technique. After the development and maintenance of hypertension for a period of five to fifteen days, the ureter of the normal kidney was anastomosed to the lumbar vein, deviating the urine from this kidney back to the blood stream. This arrangement nullified the excretory activities of the kidney but left a kidney with a presumably normal metabolism in situ. No change in arterial pressure followed this procedure for a few days. In several animals the ischemic kidney responsible for the hypertension was removed within 48 hours after the anastomosis, using ether anesthesia, and the blood pressure following this procedure was found to fall to the normal level within seven hours. Thus the kidney still in situ had maintained its ability to destroy the pressor principle at a rapid rate, differing in no way from a kidney without the ureter-vein anastomosis. In twenty to forty hours, mild hydronephrosis of the remaining kidney was sometimes sufficient to cause a slight rise in pressure (cf. Megibow, Friedberg, Rodbard and Katz, *Proc. Soc. Exper. Biol. Med.*, in press), and this persisted until the animal was sacrificed or died in uremia.

These results suggest that the chemical mediator of renal hypertension is destroyed at a rapid rate by the metabolic activity of the normal kidney, the excretory activity apparently playing little or no role in this action.

The importance of the sympathetic nervous system in maintaining the circulation during ether anesthesia in dogs. WALTER S. ROOT and FERDINAND F. McALLISTER (by invitation). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

The importance of the sympathetic nervous system during ether anesthesia was demonstrated by studying the effects of this anesthetic on the blood pressure, heart rate and plasma volume of 8 sympathectomized dogs. Mean arterial blood pressure was recorded with a Hg manometer attached to a cannula inserted in the femoral artery under local anesthesia; plasma volume was measured by Gregersen's dye method (Gregersen et al., 1935, 1937, 1938, 1939); jugular blood ether concentration was determined by the method of Ruigh (personal communication).

Recently sympathectomized dogs (2 to 3 weeks) are extremely sensitive to ether and may die during the induction of anesthesia. For this reason, we have complete data for 1 hour of anesthesia on only 4 animals. The cause of this sudden death is a precipitous fall in blood pressure accompanied by a slowing of the heart which may amount to complete asystole for many seconds. After the excitement stage, deepening the anesthetic level no longer causes cardiac slowing. The latter, which is a vagal effect, is not the sole cause of the fall in pressure for after bilateral cervical vagot-

omy the induction of anesthesia still causes as great a drop in blood pressure. Under these conditions the heart rate may remain within 6 beats per minute of the control value. During surgical anesthesia, the blood pressure continues to remain low so that at blood ether concentrations of 100 to 130 mgm. per cent the mean level of arterial pressure ranges between 30 to 50 mm. Hg. At these same blood ether levels, normal dogs show pressures ranging from 80 to 120. Since it has been shown that a similar blood pressure response occurs in dogs with low cervical spinal cord sections (Root and McAllister, 1938), it is apparent that the spinal sympathetic centers alone are not sufficient for the maintenance of the normal blood pressure level. As in high spinal dogs, sympathectomized animals do not show the decrease in plasma volume commonly found in etherized normal dogs (McAllister, 1937, 1938; Bollman, Svrbely and Mann, 1938).

The length and variability of adolescent menstrual cycles. ROZELL RORK (by invitation) and F. A. HELLEBRANDT. Department of Physiology, University of Wisconsin, Madison. (Read by title.)

Only a relatively few investigators have studied the menstrual rhythm of normal adolescent girls and little accurate data concerning its variations are found in the literature. In the present study, 231 high school students, averaging fifteen and one-half years in age, kept calendar records of the menstrual rhythm. These were collected at the end of each cycle. A total of 1690 menstrual cycles were available for the determination of *inter-individual* differences. To study *intra-individual* irregularities all those who had been observed at least nine months were selected. This group was composed of 113 cases and 1192 menstrual cycles. The extensive variability encountered made it impractical to classify the menses into types according to cycle lengths. The age at menarche, obtained by questioning, showed that the majority of girls had been menstruating three years, two for as long as seven years, and five began during the period of observation.

The menstrual cycles ranged from 8 to 122 days in duration with a mean of 31.9 and a standard deviation of ± 8.55 . The frequency histogram was skewed. More cycles were longer than the average. Corresponding results for the selected group were practically identical with those presented above. When the average length of the menstrual cycles of each girl in the selected group was studied, the scatter of the data was markedly reduced, the mean periods now falling between 24 and 53 days. With increasing catamenial experience the mean cycle length and the variability both tended to decrease. There was a slight indication that girls with a shorter average menstrual period are more regular than those with a longer one.

Blood and tissue histamine during rabbit anaphylaxis. BRAM ROSE¹ (introduced by J. S. L. Browne). Royal Victoria Hospital, Montreal, Canada.

It has been shown that a marked decrease in the total histamine content of the blood occurs during anaphylactic shock in the rabbit (Rose and Weil). No change in the plasma histamine was noted. Using the same

¹ Aided by a grant from the Banting Research Foundation.

methods, further studies have been carried out on the same species. The animals were sensitized to horse-serum and the shock dose was injected two to four weeks after the last sensitizing dose. Blood histamine was determined by the Code modification of the Barsoum and Gaddum method. Tissue histamine by that of Best and McHenry. All assays were carried out on the atropinized guinea-pig ileum preparation.

It was noted that the decrease in the blood histamine occurred within 30 seconds of the injection of the shock dose, was complete in one minute and was maintained for three to four hours. There was no correlation between the severity of the symptoms and the degree of blood histamine change. The blood histamine of control animals was unaltered following the injection of a similar dose of horse serum.

Since 60 to 80 per cent of the blood histamine is held within the white cells and since a marked decrease in these occurs during anaphylactic shock in the rabbit, it was thought that they might accumulate rapidly in some organ. Various tissues from animals in acute anaphylactic shock were removed and analysed for their histamine. No change in the histamine content of these tissues could be detected as compared to that of tissues removed from control animals.

Variation of the concentration of the acidity of the gastric contents in normal subjects before and following immersion of hand and entire body in water at various temperatures. GRACE M. ROTH and MILTON A. GABRIELSON (by invitation). Section on Clinical Physiology, Mayo Clinic, Rochester, Minn., and School of Education, New York University, New York City.

In 1932 Horton and Brown, by immersion in water at 10°C. of one hand of each of several patients who were hypersensitive to cold, produced a curve of gastric acidity similar to that produced by the administration of histamine. In 1937, Horton and Roth demonstrated that the rise in gastric acidity produced by this exposure to cold approximated a subcutaneous injection of 0.4 mg. of histamine.

Because of the tremendous amount of swimming carried on in the spring, summer and fall, the effect of the immersion of the entire body up to the neck in water at temperatures ranging from 65°F. to 108°F. on the gastric acidity of five normal subjects was studied. Two of these subjects swam on the average of five or six times a week; the other three not more than three or four times during the course of the summer.

Immersion of one hand in water at 10°C. caused only a slight rise in the gastric acidity. In 90 per cent of cases immersion of the entire body in cold water between 65°F. to 85°F. caused a definite rise in the gastric acidity fifteen minutes after the removal of the subjects from the water. Water at a temperature close to and above body temperature had little effect on the gastric acidity. Histaminase introduced by means of a duodenal tube thirty minutes before the immersion of the subject in water ranging between 65°F. and 85°F. inhibited the usual rise in gastric acidity.

*The anovulatory sex cycle of women.*¹ BORIS B. RUBENSTEIN.² Department of Gynecology and the Brush Foundation, Western Reserve University, School of Medicine, Cleveland, O.

¹ This work was in part supported by a grant from the Rockefeller Foundation to the Brush Foundation.

² Rockefeller Foundation Fellow.

Until recently it was not believed that menstrual cycles apparently normal in duration and frequency could occur without ovulation. During the past 15 years several papers (summarized by Hartman in *Sex and Internal Secretions*, 2nd ed., pp. 523-527) appeared presenting evidence for anovulatory menstruation. It is still generally held that anovulatory cycles are rare except in adolescence, at the climacteric and during lactation, i.e., during periods of readjustment of the reproductive apparatus.

The present report is based upon a study of 739 cycles of 101 patients and subjects distributed as follows: 110 cycles of 19 normal women, ages 22 to 35 years; 184 cycles of 16 psychoneurotic women, ages 26 to 37 years; 445 cycles of 66 patients whose complaint was sterility and whose age range was 19 to 43 years. Of the 739 cycles, 338 seemed anovulatory by the criteria employed; each patient had at least one anovulatory cycle. The patients were studied by the daily basal body temperature-vaginal smear method. An ovulatory cycle shows progressive fall in basal body temperature and progressive cornification of the vaginal smear until just before ovulation. Following ovulation the body temperature rises more than 0.5°F . in the first 24 hours, and more than 1.2°F . altogether. In the vaginal smear there is a sudden marked desquamation of cornified cells and an influx of leukocytes.

Anovulatory cycles may be of 3 general types.

1. The basal body temperature fluctuates irregularly and within a narrow range 0.5° - 1.0°F . The vaginal smears show no cycle; there is moderate cornification and persistence of leukocytes. It may be assumed that in such patients follicular development is inhibited.
2. The temperature drops gradually and rises gradually. The vaginal smears show gradually increasing cornification followed by a gradual regression in cornified cells, some aggregation and folding, persistent leukocytes. In this, the commonest type, it may be assumed that the follicle matures, but instead of rupturing it gradually becomes atretic.
3. The temperature drops gradually, rises sharply for one or two days, then drops again. The vaginal smears follow the normal pattern until the post-ovulatory phase. Then the cells in the smears regress as if the corpus luteum development were inhibited.

The electrical activity of the monkey's brain. MORTON A. RUBIN (introduced by R. G. Hoskins). The Memorial Foundation for Neuro-Endocrine Research, Worcester, Mass., and the Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

It is possible to obtain satisfactory electroencephalograms from non-anesthetized monkeys. In the waking macaque monkey, frequencies of 6 to 30 per sec. are recorded when the eyes are closed, with a predominant rhythm of 8 to 10 per sec. Amplitude may be as great as $150\ \mu\text{V}$. The mean amplitude, however, is from 50 to $60\ \mu\text{V}$. An increase in frequency and a decrease in amplitude are seen when the animal opens its eyes. These observations apply to all the major architectonic regions of the monkey's brain.

Transection of the spinal cord at level D_1 causes a sharp reduction of amplitude and an increase in frequency (from 10 per sec. to 20 per sec.). With eyes open, the electroencephalogram shows a still further increase in frequency (from 20 per sec. to 25 per sec.).

No appreciable alteration in the pattern of electrical activity is caused by

complete ablation of an architectonic region of the cerebral cortex. Conversely, electrograms from the same motor points of the two cerebral hemispheres may be quite dissimilar. These observations indicate an absence of correlation between specific function and autonomous electrical activity of the cortex.

Late recovery of sensory discriminatory ability after parietal lesions in the chimpanzee. T. C. RUCH, S. KASDON (by invitation) and J. F. FULTON. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.¹

Two chimpanzees trained in the discrimination of weight and roughness have been subjected to complete, unilateral parietal lobectomy. Periodic retesting during two and three years respectively has resulted in a marked and progressive improvement from the early postoperative state of nearly complete inability to discriminate. The residual capacity though clearly established is quantitatively slight. In one animal the weight ratio discriminated correctly in 80 per cent of trials was 1:1.25 postoperatively as compared with 1:1.21 before operation. In the second chimpanzee the residual deficit was somewhat greater, and in both animals discrimination of roughness was subject to less recovery. It is concluded that there are cortical and subcortical mechanisms capable of discriminative function other than the direct pathway represented by the medial lemniscus and spinothalamic tract, primary thalamic nuclei, and the parietal lobe. In confirmation of results of recent anatomical studies of thalamo-cortical projection systems, it has been shown that the posterior parietal lobe supports sensory functions in the absence of the direct projections to the postcentral gyrus. The posterior region is therefore a primary sensory area as well as an association area.

The effect of serum proteins on the polarographic curve. H. P. RUSCH, T. KLATT, A. J. DIRKSEN and V. W. MELOCHE (introduced by W. J. Meek). Departments of Physiology and Chemistry, University of Wisconsin, Madison.

During the course of a study on the use of the polarograph as a diagnostic cancer test, the various factors in the blood sera responsible for the characteristic curve were considered. A very close correlation was noted between the height of the curve and the level of the blood albumin. This relationship was not as marked with blood globulin. The fact that the protein wave of the polarographic curve is produced only by those proteins containing S—H or S—S groups agrees with the results obtained. The cystine content of serum albumin is about 2.5 per cent and that of globulin about 1.5 per cent. Our data demonstrates that any disease causing a low serum albumin will give a low curve. Since there are many conditions responsible for lowering the serum proteins, the test has a limited value as a diagnostic procedure.

The metabolism of amino acids and keto-acids in adrenalectomized rat kidney slices. JANE A. RUSSELL and ALFRED E. WILHELMI (by invitation). Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn. (Read by title.)

¹ This investigation was aided by a grant from the Knight Fund, Yale University School of Medicine.

In the hope of elucidating some of the differences in metabolism between normal and adrenalectomised animals a study is being made of the response of kidney slices from normal and adrenalectomised rats to certain amino acids and their corresponding keto-acids. The Warburg manometric technique has been used. The medium was Krebs phosphate buffer solution, and the substrate concentration was in every instance 0.01 M. In the absence of substrate the rate of oxygen uptake in slices from normal and adrenalectomised rat kidney was not significantly different, but the rate of ammonia production in the adrenalectomised kidney slices was 17.7 per cent less than the normal. With 1 (+) glutamic acid the increment in rate of oxygen uptake over the rate without substrate was less by 14.2 per cent in the adrenalectomised kidney slices, and the increments in rate of ammonia production differed by 28.3 per cent. With dl-alanine the increments in rate of oxygen uptake differed by 15.8 per cent, and the increments in rate of ammonia production differed by 19.5 per cent. With α -ketoglutaric acid the increments in rate of oxygen uptake differed by 21.8 per cent, and with pyruvic acid the difference was 31.6 per cent. Thirty to sixty observations were made in each group, and in every instance the differences were significant. Kidney slices from adrenalectomised rats therefore show a marked disability to deal with these substrates as rapidly as normal kidney slices. Experiments are now in progress which are designed *a*, to demonstrate the nature of the deficiency in tissue from adrenalectomised animals, and *b*, to ascertain whether similar defects are present in other tissues (liver and muscle) of adrenalectomised rats.

The influence of yeast feeding on the digestion and absorption of a complete diet. R. A. RUSSELL (by invitation) and E. S. NASSET. Department of Vital Economics, University of Rochester, Rochester, N. Y.

Jejunostomies were established in several dogs, so that when desired a catheter could be inserted into the intestine and its contents withdrawn, while normally they passed the fistula without leakage. The dogs were kept on a standard diet of Purina Dog Chow, and the test meal used in this study consisted of 100 grams of the Chow, finely ground and moistened with distilled water. The animals were placed in stalls and the catheter inserted before feeding; a negative pressure of 12 cms. of water was applied by water pump to withdraw the intestinal contents. The animals were then fed, and when food appeared it was collected at fifteen minute intervals, its volume and weight being recorded. Each sample was divided into two equal parts, one of which was boiled briefly on a water bath to stop digestion, and the other placed in a flask to be incubated until digestion was complete. All such samples were pooled for each experiment, and the reducing substance determined by the Shaffer-Hartman-Somogyi method.

After several satisfactory experiments on the basal diet, supplements of fresh baker's yeast were added. Control and yeast periods were run alternately.

It was observed that yeast shortens the time for the final appearance of food, suggesting increased intestinal motility, and increases the rates of digestion and absorption of carbohydrate.

Supplements containing the yeast equivalent of thiamin, riboflavin, nicotinic acid, B₆, and vitamins A and D, did not cause any significant increase

over the basal values; nor did ten times these equivalents produce the yeast effect. Experiments now under way on the fractionation of yeast indicate that the 50 per cent alcohol-soluble fraction may possess at least a part of the yeast activity noted.

Radioactive phosphorus as a tracer in anaerobic muscular contraction.

JACOB SACKS. Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor.

Radioactive phosphorus in the form of Na_2HPO_4 has been injected into anesthetized cats for the purpose of studying the distribution of the labeled atoms among the phosphorus compounds of muscle and the effect of contraction under conditions of oxygen deficiency on this distribution. The material was injected subcutaneously, and two hours later one gastrocnemius muscle was tetanized for 15 seconds and then frozen. The resting muscle was then frozen and the phosphorus present as inorganic phosphate, phosphocreatine, the readily hydrolyzable groups of adenosine triphosphate, and hexosemonophosphate separated and isolated. The radio-phosphorus content of each fraction was determined by measurement of the ionization rate produced in the electrometer.

In the resting muscle, the highest content of radiophosphorus was found in the inorganic P; the phosphocreatine P had one tenth as much, and the other two organic compounds showed even smaller concentrations.

In the stimulated muscle, the distribution of the radiophosphorus showed that none of the interchanges of phosphate groups had taken place which would be anticipated from the Embden-Meyerhof schema of reactions for the formation of lactic acid. The changes were similar to those found on balance in the contracting muscle, and correspond to the hydrolysis of part of the phosphocreatine and the conversion of another part to hexosemonophosphate.

It is concluded that the reactions of the Embden-Meyerhof schema do not represent the mechanism of lactic acid formation used by the intact cell, that adenosine triphosphate and phosphocreatine do not take part directly in any reaction concerned with the formation of lactic acid, and that hexosemonophosphate is not an intermediate in the formation of lactic acid in contracting muscle.

The vitamin B₆ deficiency syndrome in the rat. W. L. SAMPSON (by invitation), KLAUS UNNA (by invitation) and HANS MOLITOR. Merck Institute of Therapeutic Research, Rahway, N. J. (Motion picture demonstration.)

A motion picture in color demonstrating various stages in the development of the vitamin B₆ deficiency syndrome in the rat and the process of healing following administration of synthetic vitamin B₆.

Heart size and experimental atheromatosis in the rabbit. A. SANDERS (by invitation), L. N. KATZ, R. S. MEGIBOW (by invitation), S. CARLEN (by invitation) and J. RANSOHOFF (by invitation). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill. (Read by title.)

The relationship of cardiac hypertrophy to arteriosclerosis and coronary sclerosis is still controversial. This relationship was studied experimentally. Subintimal lipid infiltration was induced in rabbits by feeding a diet high in fat and cholesterol for three to four months. In eighteen

untreated controls, heart weight varied from 1.9 to 5.8 grams, and only 2 were above 5 grams. Twelve of the treated group showed either no lipoidal deposits or an exceedingly minimal change and had heart weights of from 2.1 to 6.6 grams, and 11 were below 5 grams. One other rabbit revealed only coronary involvement; its heart weight was 5.5 grams. The remaining thirteen treated rabbits developed very definite to marked atheromatous changes without gross valvular deformity; their heart weights varied from 4.8 to 11.2 grams and 12 were above 5 grams. Body weights of all three groups were within the same range. Pulse contour and blood pressure readings were taken in 4 atheromatous rabbits by the direct Hamilton technique, just before sacrifice. These were no different from similar determinations made in 5 normal untreated rabbits. Apparently marked atheromatosis of the aorta and coronary arteries in the rabbit produces cardiac hypertrophy.

Electrical resolution and interpretation of certain important components of the nerve action potential. OTTO H. SCHMITT (introduced by M. B. Visscher). Departments of Zoology and Physics, University of Minnesota, Minneapolis.

As a result of recent developments in thermionic technique it has become possible to obtain directly on the screen of a cathode ray tube, without intermediary calculation, patterns representing most of the important electrical quantities associated with the propagation of the nerve impulse. Some of these quantities are: 1, the current penetrating the nerve membrane at any instant; 2, the current flowing longitudinally down the axis cylinder; 3, the monophasic action potential of uninjured nerve; 4, the "amount of excitatory state"; 5, the amount of electrical energy expended in propagating the impulse, and 6, the residual displacement of ions as a consequent of the propagation of an impulse.

Several of these components have direct theoretical value toward establishing the nature of the nerve impulse, in determining, for example, the rate of development of excitation and in explaining the electrical mass transport of ions along the nerve in relation to the after potentials. Other components are more valuable as technical aids to other investigations; one, for example, permitting very rapid and exact measurement of nerve impulse velocity, another simplifying the separation of responses from several simultaneously excited fibers.

On the respiratory adjustment to prolonged diving in the seal. P. F. SCHOLLANDER (introduced by Laurence Irving). University of Oslo and Edward Martin Biological Laboratory, Swarthmore, Pa.

Diving experiments were performed on seals fastened to a board and placed in a bathtub. The animal was connected with a respiratory apparatus for atmospheric air recording direct volumetrically oxygen consumption, CO₂ output and ventilation. Blood samples were drawn from a toe artery before, during and after submerging the animal for from 9 to 18 minutes. On submerging the animals they expired deeply under water and the heart rate immediately dropped from 150 beats per minute to their normal diving bradycardia of 10 per minute. Oxygen content of arterial blood declined at nearly constant rate during diving, and the rate was not found to be accelerated by struggling. Calculated from the oxygen

stores, which are mainly in blood and myoglobin, the reserves are not adequate to provide during a dive for more than a third of the normal rate of metabolism above water. During diving only a slight rise in blood lactic acid occurs irrespective of struggling. Increase in lactic acid in blood first occurs in recovery, reaching after long and struggling dives 190 mgm. per cent. pH falls slightly during diving but may fall considerably at beginning of recovery. Bradycardia together with blood changes could be explained by assuming a nearly blocked circulation in the muscles during diving such as has been demonstrated by Irving in blood flow measurement to take place during apnoea in several divers and non divers. The excess uptake of oxygen after a prolonged dive was often strikingly small, corresponding to $\frac{1}{3}$ or $\frac{1}{2}$ of the diving time. The total lactic acid presumably formed in the muscles during diving as calculated from the recovery increase of it in the blood or from the total excess output of CO_2 is not sufficient for covering of the oxygen debt.

The relation of the height of the local action potential to the length of the shock-spike delay of the conducted response. DONALD SCOTT, JR. (introduced by D. W. Bronk). University College, London.

The local action potential has been examined, with the assistance of Mr. J. M. Ledingham, in the single crab nerve fibre and the results obtained are in agreement with those of Hodgkin (1938). Of especial interest was the relation of the height of the local action potential to the shock spike delay of the conducted action potential, since it provided information about the height of the local action potential for stimuli above threshold and also served to connect the present studies on single crab fibre with those made by Blair and Erlanger (1933) on frog nerve.

When the shock spike delay was examined in crab nerve it was found to increase both in duration and variability as the super threshold stimulus was reduced to threshold strength. This had previously been found in frog nerve. The peculiarities of this curve for crab nerve are explained by the fact that the conducted action potential starts from the point where the local action potential first exceeds a given height. Further data on crab nerve showed that the combined height of the local and conducted potentials is less in response to a threshold stimulus than in response to a stimulus above threshold, when recorded at the point of stimulation. This is interpreted as showing a decrease in the height of the local response with decrease of stimulus to threshold strength since the height of the conducted response is known to be constant.

When the stimulus was below threshold strength the height of the local action potential increased at an accelerated rate as threshold was approached at the same time that the variations in height also became most pronounced. These various data can be combined to show that the height of the local action potential follows a smooth S-shaped curve starting at 50 per cent threshold stimulus, reaching its greatest slope at threshold strength, and falling off again to a constant value for stimuli more than 20 per cent above threshold. The variations in the height of the local action potential are also greatest at threshold and decrease for both larger and smaller stimuli.

Blood-sugar concentration and exocrine pancreatic secretion in fasting dogs. V. BROWN SCOTT, H. J. BUGEL and U. J. COLLIGNON (introduced by

P. M. Harmon). Department of Physiology, Indiana University School of Medicine, Bloomington. (Read by title.)

Conflicting evidence on the effects of alterations in the blood-sugar concentration upon the volume and enzymatic activity of pancreatic secretion, and meager data on *unanesthetized* dogs with permanent pancreatic fistulae, initiated the following experiments.

The dogs with permanent pancreatic fistulae (Inlow method) were used. Secretion was collected from the transplanted major pancreatic duct by cannula or funnel. Dogs were selected which secreted enough "spontaneous" pancreatic juice for accurate measurement and analysis. The volume of pancreatic juice and its proteolytic activity were determined every 15-30 minutes throughout the experimental period. Proteolytic activity of pancreatic juice (activated by enterokinase) was estimated by formol titration. Blood-sugar determinations were made by the Shaffer-Hartman method on cadmium filtrates.

Dogs were trained to lie quietly on a table or to stand in stocks; observations were conducted 24 hours post cibum. Following a 1-2 hour control period, hyperglycemia or hypoglycemia was produced and the blood sugar, volume of pancreatic juice and its proteolytic activity were followed for at least 3 hours. Hyperglycemia was produced by the intravenous administration of 1 gram of glucose per kgm. body weight; hypoglycemia was elicited by 1 unit of insulin per kgm. body weight administered subcutaneously.

Results showed that very marked fluctuations existed in the volume and enzymatic activity of pancreatic secretion; these fluctuations were unaccompanied by alterations in the blood-sugar level. In 16 experiments marked hyperglycemia (intravenous glucose) produced no consistent alterations in the pancreatic secretion. Likewise marked hypoglycemia (subcutaneous insulin) failed to provoke significant changes in 16 experiments.

Our results show clearly that marked changes in blood sugar fail to elicit consistent alterations in the secretory volume or proteolytic activity of pancreatic juice obtained from *unanesthetized* dogs.

Periodic gastric motility and external pancreatic secretion in fasting dogs.

V. BROWN SCOTT, C. C. SCOTT and H. J. BUGEL (introduced by P. M. Harmon). Departments of Physiology, Indiana University School of Medicine, Bloomington, and University of Texas School of Medicine, Galveston.

Observations were conducted upon eight dogs, each having a pancreatic fistula and a gastrostomy. The pancreatic fistula was prepared by transplanting the major duct to the skin of the abdomen (Inlow operation), the gastrostomy by the method of Carlson. Drop-by-drop pancreatic secretion was recorded by a drop-recorder connected with a glass cannula which was introduced into the transplanted duct at each observational period. Gastric motility was recorded by a water manometer connected to an intragastric balloon. Simultaneous records of gastric motility and pancreatic secretion were obtained for periods of 2 to 6 hours with the trained dog lying quietly on a table 24 hours post cibum.

Fasting pancreatic secretion showed periods of augmentation. This periodicity was very irregular, its onset, frequency and duration were unpredictable. Periodic gastric contractions (hunger contractions) were

almost invariably accompanied by an increase in the volume of pancreatic secretion but such increase occurred during any part of the hunger period (first, middle, or last). Augmentation of pancreatic secretion, on the other hand, often occurred in the complete absence of *any* gastric motility.

Reports that fasting pancreatic secretion occurs only during periods of gastric motility (presumably hunger periods) and that such secretion is regularly periodic has not been confirmed by our investigations. Our results show clearly that while fasting gastric motility is associated with an augmentation of pancreatic secretion, secretory increases may occur without the appearance of *any* gastric motility.

A colorimetric method for the determination of vitamin B₆. J. V. SCUDI, H. F. KOONES and J. C. KERESZTESY (introduced by Hans Molitor). Merck Institute of Therapeutic Research and Research Laboratories of Merck & Co. Inc., Rahway, N. J.

The Gibbs phenol indophenol reaction has been adapted to the quantitative colorimetric determination of vitamin B₆. To increase the sensitivity and specificity of this reaction certain modifications have been introduced. Among these are the lowering of the pH from about 9 to approximately 7, the transfer of the vitamin indophenol to a butanol phase and the use of a veronal buffer. The usual borate buffer cannot be used because a complex, involving the borate ion and the 3 hydroxyl and 4 hydroxyl-methyl groups of the vitamin, is formed.

The test is performed by adding one volume of veronal buffer (pH 7.6) and 4 volumes of a butanol solution of 2-6-dichloroquinine chloroimide to one volume of the vitamin solution (pH 7.0-7.5). A blue color, showing an absorption peak at 660 m μ , develops to a maximum within 45 minutes after mixing the two phase system. The faint colors developing from solutions containing 2 to 10 gamma of B₆ per cubic centimeter are measured in an Evelyn photoelectric colorimeter using a No. 660 filter. The colors developed by solutions containing 10 to 40 gamma B₆ per cc., as measured in the Bausch & Lomb spectrophotometer, obeyed Beer's law.

The vitamin is completely removed from aqueous solution by continuous butanol extraction. It is quantitatively adsorbed by zeolite and can be subsequently eluted with 10 per cent potassium chloride. These methods are useful in concentrating very dilute solutions of the vitamin.

The production of hormones by human placental cells in continuous tissue culture. G. EMORY SEEGER and GEORGE O. GEY (introduced by C. G. Hartman). Departments of Gynecology and Surgery, Johns Hopkins University and Hospital, Baltimore, Md.

Human placental cells have been grown in continuous culture by the roller tube technique and the supernatant fluid medium has been tested for "cyonin" (placental gonadotrophic substance) and estrogen. The tissues cultured included full term and four months' placentae, chorionic tissue of ectopic pregnancies, and three hydatidiform moles.

Twenty of twenty-eight specimens of supernatant fluid tested for "cyonin" gave positive results as judged by the microscopic appearance of the immature rat ovary. It was found that the cells continued to elaborate the hormone over a period of two to six months. When the component parts of the medium were tested, consistently negative results were obtained.

Estrogen assays were performed on the supernatant fluid medium, using the vaginal smear technique and the six hour Astwood technique. In no case was there appreciably more estrogen in the placental medium than in the control medium. As the cord serum was found to vary widely in its estrogenic content, no one series of assays could be compared with another. To eliminate the possibility of a masking of the estrogenic effect by progesterone, the fluid was injected into spayed adult rats daily for one week. Both the control and the placental test rats remained in continuous vaginal estrous throughout the entire injection period by virtue of the estrogen in the medium. It was therefore assumed that there was no appreciable amount of progesterone present. To test the possibility of the production of inactive combined estrogens the fluid was hydrolyzed with 1 normal sodium hydroxide, extracted with hexane, acidified and extracted with ether. The placental fluid and the control thus treated contained approximately equal amounts of estrogen. Finally it was necessary to prove that the estrogen was not destroyed by incubation with placental tissue. Known amounts of estradiol were added to the placental culture medium and incubated over the usual four day period at 37 degrees. The fluid was then tested and the total amount of estradiol added was recovered.

It is concluded that human placental cells will produce "cyonin" (APL) but not estrogen under conditions found in continuous cultures.

The use of certain oils, fats and fatty acids as vehicles for the carcinogenic hydrocarbons. W. A. SELLE and W. C. WOELFEL (by invitation). Departments of Physiology and Public Health and Preventive Medicine, Medical School, University of Texas, Galveston.

The paper deals with the incidence and induction period of tumors of mice and rats produced by the injection of methylcholanthrene dissolved in various vegetable and animal fats and oils, and in mixtures of purified glycerides and fatty acids. The advantages and disadvantages of the lipids utilized in the search of suitable vehicles for quantitative studies of the carcinogenic hydrocarbons are discussed.

On the effect of various steroid hormones on the gonads. HANS SELYE. Department of Anatomy, McGill University, Montreal, Canada.

The fact that various estrogens, androgens and progesterone decrease the size of the gonads has been observed by numerous investigators. In previous experiments, we were surprised to see, however, that in the mouse the gonads of both sexes showed considerable atrophy when treated with estradiol alone while no atrophy was seen if progesterone was simultaneously administered (Selye, Hans and James Stevenson, Canad. Physiol. Soc. Meeting, Kingston, 1939). These observations have now been confirmed in the rat. It was found, furthermore, that in this species testosterone propionate likewise prevents the gonadal atrophy which is normally caused by estradiol in both sexes. This is all the more remarkable since testosterone leads to gonadal atrophy in either sex when given by itself in the same dose in which it prevents the gonadal atrophy caused by estrogens. The ovarian atrophy elicited by progesterone is inhibited by such doses of estradiol which in themselves cause a pronounced decrease in the size of the gonad. On the other hand, testosterone propionate fails to prevent the ovarian atrophy caused by progesterone. Desoxycortico-

sterone acetate causes but slight atrophy of the testes and ovaries if 2 mgm. are given daily over a period of 21 days. This hormone also fails to influence the gonadal atrophy caused in males and females by estradiol or testosterone propionate. The gonadal atrophy which results from chronic nonspecific damage, e.g., daily subcutaneous injections of formaldehyde solutions is not inhibited by progesterone, testosterone propionate or desoxycorticosterone acetate in either sex.

The temperature dependence of resting metabolism in amphibian nerve.

HERBERT SHAPIRO. Vassar College, Poughkeepsie, N. Y. (Read by title.)

The oxygen uptake by excised untreated sciatic nerves of the frog, *R. pipiens*, was measured in a microrespirometer at temperatures varying from 19 to 37°C. The value chosen for the resting metabolism was the fairly steady one attained after a period of equilibration at each temperature was allowed. The nerves were not immersed in fluid, but kept in a moist atmosphere, with no subsequent drying. Q_{O_2} values obtained varied somewhat from nerves of different frogs, and also in different portions of the same nerve, winter frogs being used. It was found that the oxidative activity of frog medullated nerve as a function of temperature conforms to the Arrhenius equation relating rate of chemical activity and temperature, and when plotted on the Arrhenius grid, yields a rectilinear curve with a μ value (energy of activation) of 10,800 calories. This would indicate that the "bottleneck" or slow step through which oxidations in resting nerve normally pass is one involving the transfer of hydrogen. This might be taken as evidence for the dehydrogenase-catalyzed system as the major controlling link in resting frog nerve metabolism. This would not exclude another parallel mechanism of a different nature, making a minor contribution to the total oxygen uptake.

Dark adaptation and dietary vitamin A deficiency. CHARLES SHEARD,

H. L. BAIR (by invitation) and L. F. STEFFENS (by invitation). Division of Biophysical Research and Section on Ophthalmology, The Mayo Foundation and The Mayo Clinic, Rochester, Minn.

In three normal adult subjects, the thresholds of light intensity and courses of dark adaptation of the macular areas and of regions 10 degrees above the macula were not affected significantly (less than 0.5 log unit) during the course of a dietary regimen very low in vitamin A content (100 to 300 I. U. daily) for periods of 44, 160 and 189 days respectively. These experimental results were checked and corroborated by tests made with different intensities of and exposures to the light adapting field (160 to 1500 millilamberts) and with retinal stimulus areas of 0.33, 1 and 2 degrees respectively. Other types of apparatus for photometric measurement of visual adaptation gave comparable data. The results show that, in certain normal individuals, considerable periods of deficiency in the intake of vitamin A may be maintained without producing any significant changes in rod or cone thresholds. In contrast, however, microscopic examination of the skin of one of the subjects (on a diet low in vitamin A for 189 days) showed the presence of changes (hyperkeratosis, keratotic plugging of hair follicles and so forth) which, it is believed, generally occur in late stages of avitaminosis A. Restoration to normal

cutaneous condition occurred in less than three weeks on normal diets supplemented with a fairly large daily intake of vitamin A. Dark adaptation measurements also have been made in conditions of follicular hyperkeratosis and in clinical cases of persons reporting restricted and low vitamin diets for prolonged periods. In general, in these conditions, threshold levels are above normal: high vitamin diets cause restoration to the normal range, although there are marked differences in the periods of time required.

The metabolism of vitamin C in normal and depancreatized dogs. SOL SHERRY, GERALD J. FRIEDMAN and JAMES BERKMAN (introduced by Elaine P. Ralli). Laboratories of the Department of Medicine, New York University College of Medicine, New York City.

The mechanism of the excretion of vitamin C by the normal dog kidney has been studied by means of simultaneous vitamin C and creatinine clearances. The results show that in the dog vitamin C is excreted by filtration and active tubular reabsorption. The reabsorption mechanism appears to be limited by a maximal rate so that when the vitamin is presented to the tubules by the glomerular filtrate at a rate exceeding this maximum, the excess is excreted in the urine. The average maximal rate of reabsorption in the 4 normal dogs studied was 0.52 mgm. per 100 cc. of glomerular filtrate.

In addition, the daily 24 hour urinary excretion and the plasma concentration of vitamin C was studied in normal and depancreatized dogs on diets totally devoid of vitamin C. The normal dogs excreted from 15 to 20 mgm. per kilo of body weight daily and their plasma concentration ranged from 0.55 to 0.65 mgm. per cent. The depancreatized dogs excreted from 1 to 6 mgm. per kilo and their plasma concentration varied from 0.35 to 0.50 mgm. per cent. Various types of fat were fed to depancreatized dogs in order to study its relation to the excretion of vitamin C.

Normal and abnormal variations in human cervical mucus. LANDRUM B. SHETTLES and ALAN F. GUTTMACHER (introduced by E. B. Astwood). Department of Obstetrics, Johns Hopkins University and Hospital, Baltimore, Md. (Read by title.)

Séguy, Simmonet and Vimeux first noted that human cervical mucus undergoes cyclic variation which is clearly coordinated with ovarian status. This was later confirmed by Lamar, Shettles and Delfs. The present paper extends these observations and records new observations on pregnancy, menopause, and cases of unexplained sterility. The latter two groups were studied both before and after therapy.

Under typical conditions human cervical mucus is scant, viscid, cloudy with a pH of 4.5 before the tenth and after the eighteen days of a menstrual cycle. Then the mucus presents an impenetrable barrier to spermatozoa and the occasional spermatozoon which succeeds in penetrating slightly, succumbs at once. From the ninth day on, until midcycle, the mucus undergoes marked changes becoming profuse, watery, clear and alkaline with a pH above 7.5. The mucus is also penetrable by spermatozoa at the rate of 2 to 3.5 mm. per minute and they survive within the mucus better than in semen. This status lasts for several days and then the mucus returns to its previous condition.

Early in pregnancy the mucus remains alkaline but highly viscid and impenetrable to spermatozoa. However, by the eighth week it has become acid, remaining so until mid-term, the latest sample thus far tested. It will be interesting to discover in an appropriate patient if immediately after conception the acid phase of the premenstrum is skipped.

In a sterility patient the mucus remained acid and impenetrable throughout two normal menstrual cycles despite the fact that ovulation took place as proved by pregnandiol. In a third cycle she was given daily one mgm. of stilboestrol and on the sixteenth day of therapy the mucus was watery, alkaline, and favorable to spermatozoa. In others, the proper days for artificial insemination were selected from the character of the cervical mucus.

Twenty menopausal patients were studied. All showed scant, viscid, acid, impenetrable mucus. Stilboestrol 0.1 mgm. daily caused no mucous effect, 0.2 mgm. gave a slight response, while in most 1 mgm. daily converted the mucus into a profuse, watery, alkaline, spermatozoon-penetrable secretion. This technique may prove an important guide for evaluation oestrogenic effects in the human.

Quantitative studies on uterine secretions. HSI-EN SHIH (by invitation), JANET KENNEDY (by invitation) and CHARLES HUGGINS. Department of Surgery, University of Chicago, Chicago, Ill.

No studies have been reported previously on the chemical nature of uterine secretion. In the dog, uterine fistulae were made by transection of the vagina and suture of the uterine cervix to the abdominal wall. Fluid was obtained by stimulation with pilocarpine hydrochloride, 6 mg. intravenously, but only from females in heat; epinephrin, atropin, and pituitrin did not cause uterine secretion. This fluid is opalescent, limpid, and is relatively scant compared to prostatic fluid obtained under similar conditions in the male. Variations in the fluid from 3 dogs were as follows: water 97.4-98.6 per cent; chloride 149-190 mille-equivalents per liter; sodium 140-170 m. eq.; calcium 0.9-2.5 m. eq.; and potassium 4-9.4 m. eq. Fluid was obtained by aspiration of the uterus in the rat following estrogen stimulation, and in rats and rabbits following ligation of the uterine cornua proximally and distally. Chloride was present at levels of 96-105 m. eq.

In none of these fluids were glucose and inorganic phosphate present in more than trace amounts.

Further studies on the restoration of carbohydrate oxidation to diabetic tissue in vitro in the absence of insulin. EPHRAIM SHORR, S. B. BARKER, EUGENE COHEN (by invitation) and MURIEL MALAM (by invitation). New York Hospital and Department of Medicine, Cornell University Medical College, New York City.

We have previously reported (Shorr Science **85**: 2210, 1937), that excised diabetic cardiac tissue regained the capacity to oxidize carbohydrate when kept for 10 hours at 37.5°C. in a Ringer-glucose-phosphate solution under oxygen. This phenomenon was attributed to the release of the tissue with time, from inhibitory influences carried over from the whole animal. It was interpreted as an *in vitro* counterpart of the Houssay experiment, furnishing additional evidence that carbohydrate oxidation was fundamentally independent of insulin.

These studies have been extended with the following results:

1. A similar elevation of the respiratory quotient to unity with time was observed with cardiac tissue from fed and fasted dogs. Apparently there exist in normal fed and fasted animals, as well as in the diabetic animal, factors inhibiting the extent of carbohydrate oxidation, the degree of inhibition being determined by the nutritional or hormonal state.

2. The rapidity with which restoration of carbohydrate oxidation occurs varies directly with the temperature. At 41°C. restoration was generally complete within four hours as compared with 10 hours at 37.5°C.

3. The presence of inorganic phosphate is an important, and may be a necessary, condition for the development of the reaction. When replaced by buffers such as glycine or sodium β -glycerophosphate, the rise in respiratory quotient is slight or fails to occur. The reaction, therefore, may proceed in two steps; the first, preparatory, consisting of the removal of inhibitory influences with time; the second, the actual shift to carbohydrate oxidation under the influence of inorganic phosphate.

4. Studies of several intermediaries involved in phosphate transfer showed no consistent changes in hexosemonophosphate or adenosine triphosphate during incubation. However, phosphocreatin invariably fell to low values as the respiratory quotient rose. This observation gains significance from the findings by Chambers, Chandler and Barker (*J. Biol. Chem.* **131**: 95, 1939) that there is an increased excretion of creatin during the "premortal" resumption of carbohydrate oxidation by the diabetic and fasted dog.

The refractory period in the non-conducted response of striated muscle. F. J. M. SICHEL. Department of Physiology, University of Vermont College of Medicine, Burlington, Vt. and the Marine Biological Laboratory, Woods Hole, Mass.

In the isolated skeletal muscle fibre (frog), with cut ends, the contractile mechanism can be excited to a normal type of response apparently without involving the conductile mechanism (Brown and Sichel, *J. Cell Comp. Physiol.* **8**: 315, 1936; Sichel and Prosser. *Am. J. Physiol.* **128**: 203, 1940). This non-propagated response is associated with no absolute refractory period; and the size of the response is a function of the strength of the stimulus even though, by using large electrodes, the entire length of the fibre be involved in the contraction.

During the course of an extension of these experiments in collaboration with D. E. S. Brown, use was made of the fact that non-propagated twitch-like contractions can also be obtained reversibly in the intact entire sartorius, provided the KCl content of the medium be raised to 3 to 4 times the usual concentration. If the entire muscle be stimulated under these conditions by means of massive electrodes so placed that the electrical field is at right angles to the longitudinal axis of the muscle, the entire length of the muscle will be involved in the contraction. Recorded isometrically these contractions have a normal form. They resemble the contractions of the isolated fibre preparation and differ from those of the normal muscle in that they are essentially local and non-propagated and also in that no refractory period is involved in their excitation. The absence of the refractory period was demonstrated, as in the case of the isolated fibre preparation, by stimulating with two equal

condenser discharges separated by a variable time interval. In the isolated fibre with cut ends and in the KCl-treated muscle the second stimulus always contributed something to the mechanical response; in the normal muscle there is an interval, related to the absolute refractory period, during which the second stimulus can contribute nothing. Since the response of the KCl-treated muscle is nonpropagated, its grading presumably does not necessarily involve the frequency distribution of the fibre thresholds.

Free amino acid and acid-base balance in nerve. ROBERT H. SILBER (by invitation) and FRANCIS O. SCHMITT. Department of Zoology, Washington University, St. Louis, Mo.

Previous investigations have shown that a considerable fraction of the anions of frog, squid, and lobster nerve must be in the form of organic anions. In squid axoplasm and in lobster nerve these anions were associated with very high non-protein nitrogen values and the average equivalent weight of the unidentified anions was found to be of the order of 150. Formol titrations and ninhydrin color reactions of deproteinized preparations indicated that free amino acid may supply the anions.

The organic anions can be separated almost quantitatively from minced lobster nerves by aqueous extraction in the cold. In such extracts, base binding by protein is negligible. In extracts deproteinized by alcohol in the cold, 80 per cent of the nitrogen is amino nitrogen (Van Slyke monometric method), the figure being approximately 0.28 m.eq. per gram nerve water. Values for carboxyl groups (ninhydrin manometric method) averaged 0.37 m.eq. per gram nerve water. As dicarboxylic amino acid, this could furnish approximately sufficient anions to make up the deficit of inorganic anions in lobster nerve. The fact that it is precipitated fairly efficiently from alcoholic solution by $\text{Ba}(\text{OH})_2$ supports the supposition that it is a dicarboxylic acid. The properties do not appear to be those of glutamic acid.

Preliminary investigations of frog sciatic nerve and of cow spinal cord indicate the presence of free amino acid in medullated nerves also and in quantities which may suffice to account for the deficit of inorganic anions, again assuming that the amino acid is dicarboxylic.

The presence of free amino acid in axoplasm in a concentration which may be conservatively estimated at 2500 mgm. per cent (in lobster nerve) indicates that it may be of considerable physiological significance not only in maintaining osmotic and acid-base balance but in metabolism as well.

Quantitative relation between post-pituitary extract and urinary chloride excretion. HERBERT SILVETTE. Department of Pharmacology, University of Virginia, University. (Read by title.)

It is known that a single injection of post-pituitary extract into animals leads to an increased urinary output of chlorides. The effects of repeated injections of the extract on chloride balance in white rats given intraperitoneal injections of sodium chloride solutions have now been determined. All injections and calculations are given on the basis of 100 grams body weight. Two cubic centimeters of fluid containing 1 U.S.P. unit of post-pituitary extract (Squibb) plus various amounts of sodium chloride were injected every 8 hours for a total of 6 injections. Urine was collected at the end of every 8-hour period, measured and analyzed for chlorides.

Food and water were withheld during the 48-hour period. The experiments reported herein have not as yet been extended beyond the injection of distilled water and 0.4 and 1.2 per cent sodium chloride solutions.

Changes in water excretion in these experiments were relatively unimportant. The animals given distilled water excreted 11.0 mgm. of chlorides in 48 hours on the average; while pituitary-injected animals excreted 26.8 mgm., a negative balance of 15.8 mgm. Animals receiving 0.4 per cent sodium chloride (chloride intake 29.2 mgm. in 48 hours) excreted 34.2 mgm. of chlorides; and pituitary-injected animals given the same salt solution excreted 48.8 mgm., a negative balance of 14.6 mgm. It would thus appear that the same dose of post-pituitary extract caused similar increases in the excretion of chlorides over the control levels, when either distilled water or 0.4 per cent sodium chloride solution had been injected. This is an indication that, between these limits, the excess urinary excretion of chlorides is a function of the amount of the post-pituitary extract rather than the sodium chloride injected.

The administration of post-pituitary extract to animals receiving 1.2 per cent sodium chloride solutions did not produce a negative chloride balance, for chloride excretion was maximal in both control and extract-injected animals. In order to promote the maximal negative chloride balance by the injection of post-pituitary extract, it seems necessary to limit the intake of sodium chloride.

An ultracentrifugal study of the action of some detergents on the chlorophyll-protein compound of spinach. EMIL L. SMITH¹ (introduced by Selig Hecht). Laboratory of Biophysics, Columbia University, New York City.

Extracts of spinach leaves prepared by grinding with sand and buffer solutions yield opaque green preparations which show microscopic particles in Brownian movement. The green protein sedimented by centrifuging at low speeds shows a random spread of particle sizes.

The ultracentrifugal behavior of the green protein was investigated in collaboration with E. G. Pickels in the Laboratories of the International Health Division of the Rockefeller Foundation, using both the Svedberg light-absorption method and a direct reading refractive index method. To clarify the leaf extracts four detergents were used: Na desoxycholate, bile salts (mostly Na glycocholate), digitonin, and sodium dodecyl sulphate (SDS). With the first three detergents, the chlorophyll is split from the protein, and the protein itself shows a sedimentation constant (S_{20}) of 13.5×10^{-13} equivalent to a M. W. of at least 265,000 as calculated from Stokes' law. In digitonin, the free chlorophyll sediments together with the digitonin micelle formed in aqueous solution ($S_{20} = 5.9 \times 10^{-13}$).

In the presence of SDS, the prosthetic group remains attached to the protein, but the compound is split into smaller units. In 0.25 per cent SDS, S_{20} is 2.6×10^{-13} over a pH range of 5 to 9, although at the more acid pH's the chlorophyll is converted to phaeophytin (removal of Mg from the molecule). In 2.5 per cent SDS, S_{20} is 1.7×10^{-13} , indicating a further splitting of the protein.

Probably 265,000 represents the minimum size of the protein in native

¹ John Simon Guggenheim Memorial Fellow.

form. The various detergents apparently attack the protein at different linkages. The SDS action seems to indicate that Mg plays no role in binding chlorophyll to the smaller protein units; it may be concerned in binding the larger units, since the Mg becomes extremely labile when the protein is split.

Insulin and dextrose tolerance in rats acclimatized to CO. ERMA SMITH and K. E. PENROD (by invitation). Department of Zoology, Iowa State College, Ames.

Female rats weighing 200 to 260 grams were acclimatized to CO by daily exposure for 5 to 12 weeks. Insulin tolerance was measured by determining the blood sugar level at $\frac{1}{2}$ hour intervals for $3\frac{1}{2}$ hours after subcutaneous injection of 2 units of insulin per kilogram body weight. Dextrose tolerance was measured similarly after intraperitoneal injection of 2 grams dextrose per kilogram body weight. Blood sugar readings preceding injection were lower in CO treated animals. Insulin tolerance was essentially normal. Dextrose tolerance in CO treated animals was significantly different from the controls; the blood sugar curve attained a higher level and presented a lag in return to normal.

Factors influencing bioelectric skin potentials. J. M. SNODGRASS (introduced by Hallowell Davis). The Laboratory of the Fertility Clinic of the Free Hospital for Women, Brookline, Mass.

A search for factors causing the erratic and varied results obtained in the measurement of "ovulation potentials" (Am. J. Obst. and Gynec. **36**: 733, 1938) has revealed a number of significant variables which directly modify bioelectric skin potentials.

Finger potentials (Am. J. Obst. and Gynec. **35**: 743, 1938) were determined on two separate groups of women. Each group numbered approximately twenty-five. Four separate pairs of finger potentials were obtained daily from each subject for several months. When the daily average potentials of each group were plotted, closely similar curves resulted. Such similarity between the groups must be due to common factors. The product of environmental temperature and relative humidity, when plotted to proper scale, gave a curve which resembled closely the average day-to-day potentials of the combined groups.

In general, the *right* hand is positive with respect to the left in right-handed individuals, and the *left* hand is positive to the right in left-handed individuals. This parallels in electrical polarity the temperature differences Heiser and Cohen (J. Indust. Hyg. **15**: 243, 1933) found to exist between the right and left wrists of an individual. The dominant member tends to be both warmer and electrically positive with respect to the less dominant.

The relationship of skin potential to the surface temperature was found to be reasonably stable and reproducible within certain limits over the range from 12 to 46°C.

Bazett and Sribyatta (Am. J. Physiol. **86**: 580, 1928) have shown that the pH of the blood in vivo decreases by 0.014 pH for each degree C increase in temperature. If one assumes the thermal skin potentials to be due to a pH concentration-cell system, very close agreement is found between the theoretical and observed values of E. M. F.

Observations on bioelectric skin potentials. J. M. SNODGRASS (by invitation) and H. DAVIS. The Fertility Endocrine Clinic of the Free Hospital for Women, Brookline, Mass., Department of Physiology, Harvard Medical School, Boston, Mass., and Loomis Laboratory, Tuxedo Park, N. Y. (Read by title.)

The electrical potential difference between two fingers of the same or opposite hand(s) is measured while the temperature of the beaker of saline that serves as electrode for one finger is varied slowly (about 1° per min.) between the limits of 11 and 46°C . In spite of individual differences and transient irregularities, the curve relating potential difference to temperature usually shows a fundamental linear trend that appears more clearly as the subject is made quieter and more at ease physically and emotionally. The most linear curves have been obtained on subjects who were drowsy or actually asleep.

Warming makes the finger more positive electrically. The slopes of the most linear curves range from 0.3 to 0.8 mv. per degree C, usually about 0.5 mv. per degree. Deviations from this slope are commonest and greatest below 18° and above 37° , when the finger is first immersed in hot or cold saline, or if the direction of temperature change is abruptly reversed. A slope as steep as 1.2 mv. per degree sometimes appears in the range from 35° to 45°C . Curvilinear deviations from the mean slope are sometimes considerable and rather characteristic of the individual.

Relatively abrupt fluctuations in potential are caused by emotional reactions of the subject. In addition to, or constituting part of, the skin galvanic response are changes apparently depending on alterations in blood flow. Slower fluctuations or deviations from the average trend may be due to direct reactions of the capillaries or to vasomotor reflexes in response to the thermal stimulation. The variations in blood flow change the thermal gradients and in turn the electrical potential. A cut or abrasion renders the finger 10 to 20 mv. more positive and greatly reduces its change of potential with temperature.

The fundamental linear trend is independent of vasomotor reflexes, as it appeared clearly in four experiments on the ears of two sympathectomized rabbits. In humans the trend is not significantly altered in light sleep, moderate pentobarbital anesthesia or deep ether anesthesia.

Gaseous metabolism of turtle's liver. CHARLES D. SNYDER and FRANK TYLER (by invitation). Laboratory of Physiology, The Johns Hopkins School of Medicine, Baltimore, Md. (Read by title.)

Perfused under steady states by methods previously described, both rates of inflow and outflow and pH continuously recorded, samples at points of input and output were taken at about 15 minute intervals and analyzed for gases by the Van Slyke manometric method. In all the 14 experiments, the observed volumes, reduced to standard terms, show that this organ at room temperature rarely uses more than 0.2 ml. oxygen per minute per 100 gram liver, and that addition of porcine erythrocytes up to one-tenth volume of perfusate fails to increase this rate.

One of us (F. T.) with the kind help of Dr. C. L. Gemmill observed oxygen consumption of thin slices of turtle's liver by means of a five-chamber Warburg respiration apparatus. Surviving in Tyrode's saturated with oxygen and kept at about 17°C ., the consumption rate (ml. oxygen per 100 gram liver per minute) turned out to have mean hourly

values varying from 0.185 ± 0.025 to 0.142 ± 0.007 from the first through the fourth hours inclusive. These figures approach the magnitude of those reported above for perfusion experiments.

Snyder (1938) observed that, under continuous steady-states of perfusion, inflow rate to the liver at times may not equal outflow rate and pointed out that any analysis of uptake and output of materials of an organ for short-lasting periods of time would have to take this fact into account. Calculated on this latter basis R. Q. values differ among themselves in sign and vary so greatly in magnitude that they become meaningless. If however the calculation is made on the basis of either inflow rate or outflow rate alone, then R. Q. falls within the range of classical values,—during one recent experiment by the first method R. Q. for individual readings varied from 0.39 to 1.32 (mean 0.69) whereas by the latter method R. Q.'s varied from 0.81 to 0.98 (mean, 0.92).

For methods in part see Snyder and associates, 1938, *Am. J. Physiol.* **124**: 647, 705; *Bull. Johns Hopkins Hosp.* **62**: 110, full report to follow.

The relation of fibrillation to atrophy in denervated muscle. D. Y. SOLANDT and J. W. MAGLADERY (by invitation). Departments of Physiology and Physiological Hygiene, University of Toronto, Toronto, Canada.

The gastrocnemius muscle of the albino rat starts fibrillating two to three days after the sciatic nerve is cut. Marked atrophy sets in about three days later and ten days after the denervation has been performed the muscle will have lost approximately 40 per cent by weight of its substance. The intraperitoneal administration of 0.5 gram per kilogram per day of quinidine sulphate (divided into 4 equal and equally spaced daily doses) will prevent the fibrillation in a large proportion of the rats thus treated. Fibrillation was routinely detected by electrical amplification of the action potentials of fibrillation, a method which we found to be more sensitive than visual observation of fibrillary movement. Atrophy in the denervated muscles of the quinidine treated animals was only about 15 per cent less than that in the untreated controls. Quinidine induces a pronounced lethargy when given in the dose required to prevent fibrillation. Sodium barbitone was administered in doses sufficient to give a degree of inactivity similar to that seen with quinidine and, although fibrillation was not interfered with, the reduction in atrophy was comparable to that obtained with quinidine. These results may be interpreted as indicating that the atrophy is not caused by the fibrillation.

Osmotically-active substances and the frog-heart rate. C. R. SPEALMAN (introduced by R. J. Main). Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

I have previously obtained evidence that the decrease in the frog-heart rate caused by such substances as glucose and sucrose in appropriate concentrations results from the osmotic transfer of water brought about by these substances. In the present experiments, I have studied a series of compounds of varying molecular weight in an attempt to show that only those substances which are osmotically active (because of their higher molecular weight) cause this slowing; while osmotically-inactive substances (of lower molecular weight) cause no change in the heart rate.

The sinus venosus of the frog heart suspended in Ringer's solution was the preparation used. The substances studied (in order of their increas-

ing molecular weight) were: ethyl alcohol, urea, glycerine, propylene glycol, arabinose, glucose, mannitol, and sucrose. The concentration used was 0.1 M/L (in Ringer's solution) in each case.

The first four substances (of lower molecular weight) cause no significant change in the frog-heart rate. The latter four substances decrease the heart rate approximately 20 per cent in each case. Evidence that only the latter four substances are osmotically active was obtained on the ventricle, where weighing experiments showed that only these latter substances cause a decrease in ventricular weight, when the ventricle is transferred from normal Ringer's solution to Ringer's solution containing 0.2 M/L of any one of the experimental substances.

Rhythmic stimulation of the labyrinth. E. SPIEGEL. Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa. (Read by title.)

Although it is generally believed that electric stimulation of the labyrinth yields reactions only if a constant galvanic current is employed, it could be demonstrated that a response may also be obtained, in cats, by stimulation of the labyrinth with a rhythmically interrupted current. Binaural stimulation (one electrode in each external meatus) was used as well as monaural stimulation by a concentric needle electrode which was introduced into the inner ear through the bulla ossea. The latter method of stimulation required smaller currents (1-3 ma.) than the former (up to 9 ma.) to elicit nystagmic responses of the eyeballs. The nystagmus was recorded on a photo-kymograph upon which was projected the shadow of a needle which had been fixed on the anesthetized cornea by a small rubber cap. With a low frequency of stimulation (e.g., 1-5 per second, sometimes even with a somewhat higher rate), the frequency of the nystagmus may be higher than that of the stimuli, a rhythmic after-discharge appearing in addition to the reactions which correspond to each electric shock. With a higher rate, the rhythm of the nystagmus follows that of the stimuli. A slow and fast component may be distinguished on stimulation at low frequencies. At frequencies above 10 per second the reaction assumes the characteristics of a tremor (frequencies up to 27 per sec.) which may be followed by a coarse nystagmus after cessation of stimulation.

Efferent forebrain systems involved in the production of rage reactions after decortication. E. SPIEGEL, H. R. MILLER (by invitation) and M. J. Oppenheimer (by invitation). Department of Physiology and Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa.; and Montefiore Hospital, New York City.

The rôle of the following systems in producing rage reactions in decorticated animals was studied experimentally on 55 cats and 12 dogs:

- 1, motor and premotor areas; 2, frontal pole, tractus neocortico-septalis; 3, parietal lobe (lateral surface); 4, occipital lobe; 5, lateral surface of the temporal lobe; 6, gyrus cinguli; 7, hippocampus-fornix system; 8, striatum; 9, olfactory tubercle, preoptic area, medial forebrain bundle; 10 amygdaloid nucleus.

A lesion of any of the systems 1 to 6 and 8 did not produce rage reactions. Such reactions, or at least partial reactions, e.g., running movements, struggling, panting, and emotional hyperexcitability were observed

if the lesions involved the olfactory tubercle or the amygdaloid nucleus or as, in a number of cases, an extensive part of the fornices.

Vertical nystagmus produced by lesions of the cerebellar vermis. E. SPIEGEL and N. SCALA (by invitation). Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa. (Read by title.)

Experiments on cats showed that besides the vertical nystagmus (v.ny.) appearing after lesion of the cranial part of the vestibular nuclei a second type of v.ny. must be distinguished. It is produced by lesion of parts of the lobus medianus posterior of the cerebellum (particularly uvula and nodulus of the old nomenclature). The position of the head may influence the frequency and amplitude of this vertical nystagmus (increased in side position and reclining position with vertex downward). It is suggested that this v.ny. is due to a release of the vestibular reflex arcs from cerebellar inhibition. The rôle of the cerebellar nuclei is being studied.

Studies on the metabolism of the Brown-Pearce carcinoma in normal and hypoglycemic rabbit sera. RICHARD H. STECKEL (by invitation) and JOHN R. MURLIN. University of Rochester, Rochester, N. Y.

Since Warburg was able to simulate tumor metabolism in growing tissues by means of cyanide, there has been speculation on the possibility of bringing about a reversal of such a transformation and of changing the metabolism of tumor in the direction of the normal. To this end the authors have studied the metabolism of the Brown-Pearce carcinoma in normal fasting rabbit sera and in sera prepared from the same animals reduced to the shock level with massive injections of insulin. A modified type of the Warburg respirometer was employed. Experiments to date have shown the oxygen consumption of the tumor slices in the hypoglycemic serum to be increased an average of 17% over that of slices of the same tumor in normal fasting serum. Preliminary experiments also have indicated that the anaerobic glycolysis of the tumor in normal serum is of the order of five times that in the hypoglycemic serum. Studies are now underway to determine if the effects noted are attributable entirely to the differences of glucose concentration in the two media. The direct addition of regular insulin to a medium of normal serum to the extent of 0.2 u./cc. was found not to affect oxygen consumption by the tumor.

Observations on the utilization and excretion of calcium via the colon in the adult man. F. R. STEGGERDA. Department of Physiology, University of Illinois, Urbana.

Studies were made of the amount of calcium the adult man requires in his food to maintain a balance with the calcium lost by way of the urine and feces.

The nine subjects studied first lived on a diet low in calcium for 20 to 24 days until a constant state of negative balance was reached. The diet was then supplemented with calcium in the form of milk until a state of equilibrium was obtained, from which the percentage of calcium utilization by the body cells could be calculated. The calcium was administered in various forms of milk and, with the exception of three subjects who gave a utilization of 52.4, 36.7 and 33.5 per cent respectively, the figures show a utilization of approximately 20 per cent in all cases.

With reference to calcium excretion by way of the colon, it should be pointed out that the rectum has a far richer blood supply than the colon. Therefore, it is logical to believe that greater quantities of calcium may be excreted into the rectum than into the colon. In our experiments calcium determinations of the rectal and colon ends of the stool were made. A statistical analysis of the data indicates that there is a greater excretion of calcium by way of the rectal, than of the colon, mucosa. A limited number of experiments also indicate that the pH of the rectal end of the stool is more alkaline than that of the colon end.

Fatigue and curarization in the single nerve-muscle preparation. S. E. STEIMAN. Department of Physiology, Harvard Medical School, Boston, Mass. (Read by title.)

Single muscle fibers or a small group (2-8) were isolated from the frog sartorius with intact nerve supply. The responses to indirect stimulation were recorded by the mercury globule method of Pratt. Records of stimulation at 5 per sec. over a continued period (5 min.) showed two kinds of fatigue—muscular and “end-plate.” During the first two minutes the fiber followed the rate of stimulation but the height of the contraction progressively declined. The onset of “end-plate” fatigue, however, was manifested in an all-or-nothing manner, i.e., the fiber would suddenly fail to respond to some of the stimuli. At first, this failure was intermittent and irregular, but as “end-plate” fatigue developed further, the interval between responses became greater. During the latter period the height of the contraction began to increase and approach that obtained at the beginning of stimulation.

The blocking of nerve impulses at the “end-plate” by curare also occurs in an all-or-none manner. After the addition of a small amount of curare to a preparation stimulated at slow frequencies (1 per 2 to 5 sec.) the contractions continue for a short period and suddenly cease. Acetylcholine (15-20 γ) will produce a decurarization (one or two contractions) but this is not always obtained. The stage of curarization is an important factor in producing a decurarization by acetylcholine. The optimal condition for this effect is the degree of curarization where an impulse just fails to produce a response. The exact dose of acetylcholine is difficult to determine and the point where a contraction would result is easily missed.

After eserine (0.1-0.3 mgm.) there is approximately a 100 per cent potentiation of the height of muscular responses of a normal preparation stimulated 1 per 10 sec. Stimulation at 10 per sec., after eserine, results in one or a few responses, and then no further contractions although stimulation was continued for a minute or more. Frequently, after the initial response, there would appear 10 to 15 seconds later a second contraction and none after that.

Sodium and potassium in frog muscle. H. BURR STEINBACH. Department of Zoology, Columbia University, New York City.

It is known from the work of Fenn and others that potassium is lost from muscle fibers either during activity or under conditions of low external potassium, and that sodium enters in exchange for the lost potassium. This report is concerned with the sodium-potassium exchange

in isolated frog sartorii. Potassium was removed by soaking the muscle in K-free Ringer's solution and partially replaced by soaking the K-depleted muscles in Ringer's solution containing 0.01 n potassium. Sodium and potassium were determined on the same muscles, chloride on other muscles treated to exactly the same experimental conditions. As was expected, sodium content, plotted against potassium content of muscle shows a linear inverse relationship. Over half of the potassium can be replaced by sodium with no corresponding increase in chloride content, indicating an ion exchange. With greater losses of K, the chloride space sometimes increases.

Reversibility of this exchange is shown by the following experiment: Muscles soaked 17 hours in K-free Ringer's contained 3.67 meq per cent K, 5.65 Na and 2.89 Cl. The mates to these muscles, soaked 17 hours in K-free Ringer's and then 8 hours in Ringer's containing 0.01 n K, on analysis showed 5.67 meq per cent K, 3.35 Na, and 2.86 Cl. Average figures for other muscles from other animals soaked only two hours in K-free Ringer's were 7.10 meq per cent K, 3.31 Na and 3.38 Cl. It is clear that muscles can take up potassium without chloride even after losing at least half of their original potassium content. In these experiments the muscles remained irritable.

The magnitude of the injury potential is dependent, in part, on the potassium content of the muscle fibers. Other factors are apparently operative however which prohibit an exact evaluation of the rôle of intracellular potassium in the production of the injury potential, although in general the measured potential difference is lower as the K concentration of the fibers decreases.

A device for measuring vasomotor reflex time and some preliminary observations on vasoconstrictor time. ARTHUR H. STEINHAUS, DAVID E. MISNER (by invitation) and CARL W. SAUBERT (by invitation). George Williams College, Chicago, Ill.

We have developed an instrument to record the time which elapses between the application of a temperature stimulus such as cold water to one hand and the reflex response of the bloodvessels in a finger of the other hand. This interval of time we have called vasomotor reflex time. The instrument is a modification of the recording plethysmograph of Johnson (Johnson, C. A. J. Lab. and Clin. Med. 17: 59, 1931; *ibid.* 1932; Surg. Gyn. and Obst. 55: 731) to which we have added a timing device recording $\frac{1}{2}$ second intervals and an electromagnetic signalling device for marking the moment of stimulus application.

Out of a limited experience with this device we can report the following:

1. In a series of readings on fifty different subjects we have found the vasoconstrictor reflex time to range from $\frac{1}{2}$ second to 6 seconds.
2. We have found some indication of shorter times in persons who regularly take cold baths compared with those who do not; but we are not yet able to say if the cold baths are in any way causally related to shortening of this time.
3. We have also some indication that certain other changes in bodily condition may be paralleled by transient changes in this time.

Though at this writing (January 8) we have insufficient data to present a statistical expression of the reliability of this method it appears that we can attain an accuracy of about $\pm \frac{1}{2}$ second.

*The effect of drugs on the pulmonary arterial pressure in the trained unanesthetized dog.*¹ F. S. STEINITZ and L. FRIEDBERG (introduced by L. N. Katz). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

The action of drugs on the systemic circuit has been studied recently in our laboratory (Katz and Friedberg, *Am. J. Physiol.* **127**: 29, 1939) on trained unanesthetized dogs. We have also investigated the pulmonary pressure in trained unanesthetized dogs by means of a special pulmonary cannula (Katz and Steinitz, *Am. J. Physiol.*, **128**: 433, 1940). Simultaneous pressure readings were obtained from the systemic and pulmonary systems with Hamilton monometers.

Since we have found that the pulmonary pressure does not change in experimental renal systemic hypertension (Katz and Steinitz, *ibid.*), we have analyzed the acute effect on the pulmonary and systemic pressure following renin injection. We have also studied the action of a number of other drugs.

The injection of pitressin intravenously resulted in a rise in systemic arterial pressure with no immediate effect upon the pulmonary arterial pressure. However, when the heart began to fail, as shown by a fall in systemic pressure and a drop in pulse pressure while the heart rate slowed, a rise in the pulmonary pressure occurred. This suggested a congestion of blood in the lung due to the predominance of left heart failure over that of the right. The pulmonary pressure rose from 20/7 to a level of 50/40 mm. Hg. As the heart recovered from the depressant effect of the drug, the systemic blood pressure rose again and the pulmonary pressure fell again towards the normal level. The elevation of systemic arterial pressure which was sustained at this stage was not accompanied by any pulmonary arterial pressure elevation.

Adrenalin caused a rise in the arterial pressure in both circuits. Acetylcholine caused a fall in systemic arterial pressure with no change in pulmonary arterial pressure. Preliminary observations have also been made on the action of aminophylline and amyl nitrite.

Our results can be explained on the basis that in the unanesthetized dog the pulmonary bed is primarily a passive system; its pressure being dependent chiefly upon the balance in the output of the two ventricles.

*The effect of ammonium chloride on pain.*² W. B. STEWART (by invitation), B. D. JUDOVICH (by invitation), JOSEPH HUGHES and A. WALTI (by invitation). Philadelphia General Hospital, Institute of the Pennsylvania Hospital and Research Laboratories, Merck and Company, Rahway, N. J.

It has been observed clinically that many cases of root pain and of intercostal neuralgia have been relieved by paravertebral injection of an extract of pitcher-plant root (*sarracenia purpurea*). After the injection of this extract there results relief of root pain and loss of the hyperesthesia usually observed in the distribution of the dermatomes supplied by the roots involved, with preservation of superficial sensibility including pin prick. From the alkaline distillate of the pitcher-plant root, a white

¹ Aided by the A. D. Nast Fund for Cardiac Research.

² Aided by a grant from the John and Mary R. Markle foundation.

crystalline substance was isolated which possessed these same clinical properties. This substance was identified as ammonium chloride.

Immersion of the isolated saphenous nerve of the cat both in pitcher-plant extract and in an optimal concentration of ammonium chloride results in the selective abolition of the activity in "C" fibers. This is interpreted as being corollary evidence of the duality of pain sensation.

A perfusion method for suspensions of microorganisms. THEODORE J. B. STIER. Biological Laboratories, Harvard University, Cambridge, Mass. (Read by title.)

The main difficulty in using diatomaceous filter candles in a perfusion method for microorganisms is the rapid clogging of the filters when suction is employed continuously. We have circumvented this difficulty by periodically interrupting the vacuum and applying a positive pressure. In our apparatus, a small volume of the perfusion fluid is trapped within the filter outlet tube with each application of the pressure. This fluid is then forced through the filter in the reverse direction, thus washing off the layer of material which accumulates on the outside of the filter during the vacuum portion of each cycle.

The filter candle dips into the suspension and is attached above the reaction chamber to one of the short arms of a Y-tube which is held in the inverted position. The vacuum line is attached to the other short arm of the Y-tube. Two solenoid valves, actuated by an electric clock through a relay system, control the application of vacuum and pressure to the filter. The cells are kept in suspension by a small motor driven stirrer. For suspensions of bakers' yeast, a single $2\frac{1}{2} \times \frac{5}{8}$ in., 6 lb. Mandler filter with 20 sec. vacuum (ca. 500 mm. Hg) followed by 40 sec. pressure (6 lb.), usually gives a perfusion rate of about 1,500 ml. per hour when a 5 per cent dextrose solution is employed. The rate of perfusion can be modified by altering the magnitude and duration of the vacuum, the porosity of the filter and the total filtering surface in the reaction chamber. Sintered-glass filters are unsatisfactory for yeast suspensions under these operating conditions. After a relatively short time they become clogged and then break at the filter-plate junction.

The method was developed for studies of carbohydrate and lipid assimilation and cell multiplication of yeast, where a constant composition of the suspending medium should be maintained throughout the course of an experiment and where secondary effects produced by the by-products of metabolism should be reduced to a minimum. The perfusion apparatus has also been especially useful for concentrating pure cultures of yeast and washing the cells under sterile conditions at a low temperature.

*Some observations on pH, lactic acid and phosphates of the cortex.*¹ WILLIAM E. STONE (introduced by J. G. Dusser de Barenne). Laboratory of Neurophysiology, Yale University, School of Medicine, New Haven, Conn.

The relations of pH, lactic acid and various acid-soluble phosphate fractions of the cerebral cortex were investigated in cats under Dial (Ciba) anesthesia and artificial respiration. Some of the animals were also curarized. The pH of the cortex was measured as described in previous

¹ Aided by a grant from the Child Neurology Fund (Friedsam Foundation).

publications from this laboratory; the lactic acid and phosphates were determined after local freezing of the brain *in situ* with liquid air.

The following results are of interest here: 1. The freezing and extirpation of a relatively small area of the cortex results in a marked increase in the lactic acid of adjacent areas. 2. The freezing and extirpation of a large area of the cortex and subjacent structures results in a marked increase in the lactic acid and a marked decrease of the pH of the cortex of the contralateral hemisphere, while only small, probably insignificant, changes in blood lactic acid occur. The animals under 1 and 2 were kept under constant artificial respiration. 3. From observations 1 and 2 it follows that not only the CO_2 -tension of the blood (as shown before) but also lactic acid of the cortex is a factor in regulating the pH of the cortex. 4. The phosphate fractions determined and their normal average values under the prevailing experimental conditions were: inorganic P 11.1 mgm. per 100 gram tissue, phosphocreatine P 11.9 mgm., pyrophosphate P 13.0 mgm., and hexosephosphate P 21.8 mgm. It was found that the increase in lactic acid content of the cortex was accompanied by a slight decrease in phosphocreatine P and a corresponding increase in inorganic P. No uniform changes in the other phosphate fractions were observed.

Studies of the action of methadren; methyl-adrenalin. J. W. STUTZMAN and O. S. ORTH (introduced by W. J. Meek). Department of Physiology, University of Wisconsin, Madison.

Studies concerning the relationship of chemical configuration to production of cardiac arrhythmias in 11 primary and secondary sympathomimetic amines during anesthesia have been reported previously. The opportunity of using methyl adrenalin, "methadren," (3,4-dihydroxy-phenyl α -dimethyl amino ethanol) in similar experiments allowed the extension of such tests to a tertiary amine and seemed to warrant further studies of its physiological actions. This report deals with pressor, bronchiolar, gastro-intestinal, and glycogenolytic activity, cardiac automaticity, duration of infiltration anesthesia, and toxicity of methadren.

In the nembutalized rabbit 25 to 30 times as much methadren as adrenalin injected intravenously gave comparable pressor effects. In the dog a higher ratio of 30 to 50 times as much methadren was required, both in the unanesthetized spinal animal and those under surgical cyclopropane, ether, or vinylene anesthesia.

In the dog under ether there were no cardiac irregularities and under cyclopropane and chloroform fewer irregularities than in the control experiments with pressor dosage of 0.5 mgm. per kilogram of methadren. Thus cyclopropane does not enhance the activity of this tertiary amine on the automatic tissue of the dog's heart as has been shown previously to occur with adrenalin or other primary or secondary sympathomimetic amines having the catechol nucleus.

Both in the isolated perfused guinea pig lung and the intact anesthetized dog, bronchiolar spasms from histamine could be relieved by a pressor dosage of methadren comparable to adrenalin.

Twenty-five to forty times as much methadren as adrenalin in 1 per cent or 2 per cent procaine gave equivalent prolongation of local anesthesia of the rabbit's eye.

Intravenous methadren in the dog gave less pronounced and less pro-

longed glycogenolytic response than the comparable pressor adrenalin dosage. Subcutaneous injection in the rabbit gave a comparable rise of blood sugar but of shorter duration.

For rats the MLD_{50} for methadren per kilogram was found to be subcutaneously 105 mgm., intraperitoneally 50 mgm., and intravenously 5 to 6 mgm. For rabbits the figures are 25 to 30 mgm., 20 to 25 mgm., and 2.50 to 3.75 mgm., respectively. In pups the intravenous toxicity was found to be between 10 to 15 mgm. and in adult dogs 7.5 mgm. per kilogram.

Abnormal thirst in diabetes insipidus. H. G. SWANN (introduced by V. Johnson). Department of Physiology, University of Chicago, Chicago, Ill.

A total nephrectomy prevents the abnormal fluid intake of diabetes insipidus under all conditions except one: when the animal (rat) is in the permanent phase of diabetes insipidus and given solutions of NaCl to drink. Under these conditions, an exaggerated fluid intake continues, resulting in great weight increases and edema. This suggests that an abnormal thirst, in some way connected with abnormal NaCl metabolism, is responsible for the permanent, but not the transient, phase of diabetes insipidus. This hypothesis receives further support from other experiments. It has been shown that the permanent phase of diabetes insipidus is dependent almost entirely upon the NaCl taken in by the animal. It has also been observed in a few animals with diabetes insipidus that saline solutions are ingested at times at rates exceeding the ability of the animals to excrete the ingested fluid.

Arrangement of visual field on cat's cortex. S. A. TALBOT (introduced by W. H. Marshall). Laboratory of Physiological Optics, Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital, Baltimore, Md.

Potentials at a point on the striate cortex show greatest amplitude and shortest latency when a small brief test-flash comes within 2° of the optimal locus in the visual field. A moist thread on the exposed pia mater or an insulated needle reaching the buried surfaces of the cortex yields reproducible localization for several hours, if the cornea is kept moist and the eye immobilized by atropin and a wire ring. While deep anesthesia (nembutal) and dark adaptation affect localization but little, they are needed to measure the weaker responses coming from a given test-brightness in peripheral vision. A sufficiently bright flash, subtending $20'$ of arc and properly placed, elicits 100 microvolts or more from any functional striate point.

By this method, the left half of each retina projects to the left cortex, and right to right; the lower field is forward on the brain, the upper field occipital. Central vision projects to the dorsal surface just forward of the apex of the tentorium. The vertical meridian of vision extends forward about 15 mm. from here along the midline, and backward along and just rostral to the s.lateralis posterior. The upper right quadrant of the field projects to the left g.compositus medialis, with the extreme right periphery around the lateral tip of s.lateralis. The lower right quadrant projects to the medial wall of the left marginal gyrus, with periphery along the upper lip of s.splenialis. The right horizontal meridian follows the lateral

limb of s.splenialis inward toward the midline, then upward to the center of gaze described above. The upper and lower left quadrants and their dividing meridian project correspondingly on the right cortex.

The surface resolution for central vision is about 2° per mm., but for 30° below gaze is 5° per mm. of dorsal cortex. The lateral periphery is also diffusely projected. Across the dorsal surface of the marginal gyrus there exists a clear lateral discrimination which is anomalous.

Multiple responses in the optic tract and optic cortex of cat. S. A. TALBOT (by invitation) and W. H. MARSHALL. Laboratory of Physiological Optics, Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital, Baltimore, Md. (Read by title.)

A monopolar or concentric bipolar macro-electrode in the optic tract of the cat shows potentials with much detail, upon stimulation of the retina by a flash of light. These details in the optic tract are closely related to the multiple cortical responses to a single brief flash. Tract potentials are elicited by general illumination of the retina or by local photic stimulation. Their timing, amplitude and complexity can be varied systematically by change in brightness and duration of the flash, transient and steady adaptive states, and depth of anesthesia.

The tract responds to strong photic stimulus by a primary wave at about 30 msec. followed closely by one or two subsidiary waves. The spike activity is depressed for about 90 msec. during an electrode-negative recovery phase. Then appears a burst of spikes, followed by a train of slow waves (30 to 50 cps.) often lasting 200 msec., waxing and waning. Such oscillations also appear spontaneously under certain conditions of adaptation. A stimulus seems to break up the existing pattern by a new series, starting at the primary response and governing the subsidiary waves and bursts.

The individual multiple-response waves of the cortex react selectively to decreasing stimulus, to deep transient anesthesia (intravenous nembutal), and to convulsants (local picrotoxin). Changing adaptive level alters their spacing by discrete steps equal to the period of the slow oscillations in the tract. Under the conditions employed, both the main and subsidiary cortical waves occur only at spacings which are a multiple of this unit. The second large wave, the primary cortical wave and its subsidiaries all correspond to definite waves in the tract response, and so obviously originate prethalamically.

Simultaneous records also show that small "spontaneous" periodic activity under nembutal in the optic cortex (alpha rhythm) is submultiple and parallel to the "spontaneous" oscillations in the tract described above.

A study of the pharmacological action of ephedrine sulphate on the turtle heart. H. L. TERRY (introduced by Harold N. Ets). Department of Physiology and Pharmacology, Loyola University, Chicago, Ill.

A study of the action of ephedrine sulphate was conducted on large turtle hearts, in situ, perfused by way of the left lateral vein.

The influence of buffers in the perfusate was studied. A solution containing sodium chloride 0.7 per cent, potassium chloride 0.03 per cent, and calcium chloride 0.026 per cent buffered to pH 7.4 with monobasic sodium phosphate 0.001 per cent and sodium bicarbonate 0.02 per cent, maintained normal rhythm and tonus phenomenon throughout experi-

ments of ten hours. In the absence of buffers the rhythm of the heart became irregular, the tonus waves disappeared and the general tone level diminished within the first hour.

In all records, before instituting an experimental procedure, a normal tracing of an hour's duration was obtained. The stimulating or sympathomimetic action of weak ephedrine solution (1:10⁵ to 1:10⁸) was diminished and the reversal or pituitrin-like effect of strong ephedrine solutions (1:50,000 to 1:500) was completely lost in the absence of buffers.

A study of the osmotic effects of ephedrine sulphate, relative to its reversal of action, was made by determining the isotonic blood concentration of the ephedrine salt with the empirical formula $[(C_{10}H_{15}ON)_2H_2SO_4]$. Cryoscopic determinations revealed that a 3.5 per cent solution of this substance was isotonic with a 0.7 per cent solution of sodium chloride. The stimulating effect of weak ephedrine solutions and the reversed action of strong ephedrine solutions, occurred whether or not isotonicity was adjusted by proportionate reductions of sodium chloride to compensate for the ephedrine salt added.

Concentrations of 1:10⁵ to 1:10⁸ of ephedrine sulphate had a sympathomimetic action on the turtle heart and acted synergistically to prolong epinephrine stimulation. Strong ephedrine solutions, i.e. 1:10³, suppressed the fundamental beat and antagonized epinephrine stimulation. This antagonism was not abolished by perfusing the heart with a 1:10⁴ solution of atropine sulphate. Cocaine hydrochloride in a solution of 1:10⁴, augmented the reversal effect of strong ephedrine solutions and antagonized the sympathomimetic action of weak ephedrine solutions.

Bilateral synchronization in the human electroencephalogram. PER OLOF THERMAN (introduced by Hallowell Davis). Department of Physiology, Harvard Medical School, Boston, Mass. (Read by title.)

Bilateral electroencephalograms of 30 medical students were recorded under standard conditions with a two-channel ink-writer and studied for the synchronization of similar waves from corresponding areas (frontal, precentral, and occipital) of the two hemispheres. The frequency bands centering at 10 and 3 cycles were also selectively studied with the aid of broadly tuned filters placed in the recording circuit. Monopolar records (reference electrodes on both ear lobes) and bipolar transcranial records (electrodes on corresponding points, right and left) were taken alternately.

The bipolar transcranial record shows complete absence of some waves and a decrease in amplitude of other waves as compared with the monopolar records. At least part of the difference is due to synchronization of activity at corresponding points, and its consequent cancellation in the bipolar record.

A simple rectifier system was employed to measure in arbitrary units the integrated electrical output from the amplifier during periods of one minute. We treated statistically the results obtained from the ten students whose records were most normal, self-consistent, and freest from evidence of approaching drowsiness. The "monopolar output" from corresponding points in the frontal and precentral regions is nearly identical for the two hemispheres (± 5 per cent in unfiltered as well as filtered recordings), and the "bipolar" electrical output is consistently less than the "monopolar." In the occipital region, greater variability appears between the two sides, especially with respect to the 10-cycle rhythm. When the

10-cycle filter is placed in the circuit, the bipolar connection gives the same output as the monopolar within the limits of probable error. It also appears that the waves in the 3-cycle band are best synchronized in the pre-central region and the 10-cycle waves best in the frontal region. Both types of wave are least synchronized in the occipital region, but the 10-cycle waves far less than the 3-cycle waves.

The removal of blood lactate after exercise. JESSELENE THOMAS (by invitation) and ANCEL KEYS. Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis.

Young women subjects exercised on a bicycle ergometer for 1 to 2 minutes at rates of 6000 to 8500 foot pounds per minute and then "recovered" under 3 conditions in different experiments: 1, bed rest; 2, continuing mild (sub-lactate producing) exercise on the ergometer; 3, bed rest with increased circulation in the legs produced by diathermy. Blood lactate and total circulation rate were measured at intervals during the recovery period. In 11 experiments the velocity constant of lactate removal was 0.0531 in bed rest. A simple relationship between maximum height of lactate level and the velocity constant was not found regularly. It was rarely possible for these women to attain blood lactate levels comparable with the maxima reported for men.

Continuing mild exercise during recovery produced an increased rate of lactate removal in two subjects but not in a third. The speed of lactate removal was not a simple function of the total circulation in any of the subjects. In two subjects diathermy produced a decreased rate of lactate removal but the third subject showed an increased velocity constant. Both mild exercise and diathermy produced marked elevations of the minute output in all subjects. The minute output was roughly proportional to the metabolic rate in mild exercise, whereas diathermy only slightly raised the metabolic rate. Repetitions showed that one subject consistently differed from the others in response to both mild exercise in recovery and to diathermy.

*A study of carbohydrate metabolism in experimental adrenal insufficiency and in patients with Addison's disease. Effect of desoxy-corticosterone acetate treatment.*¹ GEORGE W. THORN, GEORGE F. KOEFF (by invitation), DANIEL KUHLMANN (by invitation)² and ELIZABETH F. OLSEN (by invitation). Johns Hopkins Hospital, Baltimore, Md.

Adrenalectomized dogs. Fasting respiratory quotient, respiratory quotient following glucose administration, blood sugar curve following glucose given orally, and the effect of insulin and epinephrin on the blood sugar level were determined in four adrenalectomized and six normal dogs. All animals were fed the same diet. The adrenalectomized dogs were maintained in excellent condition with implanted pellets of desoxy-corticosterone acetate.

No significant difference was observed in the two groups of animals in the values for fasting blood sugar, blood sugar curve following glucose and fasting respiratory quotient. Adrenalectomized dogs maintained on desoxy-corticosterone acetate were more sensitive to insulin than normal

¹ This study was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

² Rockefeller Fellow from University of Strasbourg, France.

dogs. Large doses of desoxy-corticosterone and adrenal cortical extract failed to alter significantly the insulin sensitivity. Epinephrin administered at the same time as insulin counteracted the hypoglycemic effect of the latter.

Addison's disease. Glucose (0.5 gram per kgm.) was injected as a 20 per cent solution over a period of 30 minutes. This rate of glucose administration approximates the maximum rate of intestinal absorption and hence provides a test which is within physiological limits. The blood sugar curve following the intravenous administration of glucose was compared with the curve following glucose given orally.

Fasting respiratory quotient and non-protein respiratory quotient were determined during a period in which patients were maintained first on a diet of 100 grams of carbohydrate and later on a diet of 400 grams of carbohydrate, protein and total calories remaining unchanged. Oxygen consumption and respiratory quotient were studied following the administration of glucose and following a protein meal.

The fasting respiratory quotient of *untreated* (hormone) patients rose rapidly following the increased ingestion of carbohydrate.

The low blood sugar curve following intravenously administered glucose indicates that delayed or impaired intestinal absorption of glucose does not necessarily explain the flat type of curve observed in Addison's disease. An inability to regulate the blood sugar level is indicated by the hypoglycemia observed 2 to 4 hours following oral and intravenously administered glucose. Desoxy-corticosterone acetate treatment does not correct this disturbance although continued synthetic hormone therapy is associated with some improvement in the oral glucose tolerance curve which may be accounted for by the improved clinical condition.

Preliminary studies of the reaction of the tissues to intravenous injections of lipoids. I. The reaction to the ether insoluble fraction of beef brains.

EDNA H. TOMPKINS (introduced by Ann S. Minot). Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn.

The material for these studies was obtained by extraction of a crude mixture of brain lipoids donated by Dr. David Klein of the Wilson Laboratories. The ether insoluble material consists of a mixture of the galactolipides and the sphingomyelins.

The material was injected daily in rabbits in emulsions in 5 per cent glucose. The amount of injection was increased gradually until brought to a stationary level varying from 0.05 gram to 0.7 gram daily. Blood counts were made by the supravital technique throughout the period of injection. The tissues were studied supravitaly at the time of sacrifice, and also fixed for sectioning.

The animals tolerated the injections well. A continued elevation of the white count, accompanied by an increase of both the polymorphonuclear cells and the lymphocytes, developed soon after the injections were begun. Cells like those to be described in the tissues were never met in the blood.

The findings in the tissues varied with the amount of material given and the length of time over which it was given. The spleen was usually enlarged and of a firm consistency and pale in color. The livers were usually pale but normal in consistency. The supravital smears of the spleen, and to a less extent of the other reticuloendothelial organs, con-

tained many very large macrophages which were characterized by large uniform vacuoles. These were either unstained or dark red in color. The macrophages of the lung were atypical. Large foam cells were found in nests in the fixed sections of the spleen, or as massive infiltrations of the reticulum. Similar cells were found infrequently in the sections of the other organs. There was no proliferation of fibrous tissue.

The sections of the spleen were similar to those described in cases of Gaucher's disease. When stained supravital cells from the tissues were similar to those obtained from subcutaneous areas injected with mixtures of sphingomyelin and cerebrosides. When fixed, they were similar to the fixed cells from the subcutaneous areas, and also to the cells described in cases of Gaucher's disease and Niemann Pick's disease.

Urine excretion during anoxia from normal and denervated kidneys in dogs with and without adrenal glands. LOUIS A. TOTH (introduced by Henry Laurens). Laboratory of Physiology, Tulane University School of Medicine, New Orleans, La.

The elimination of the adrenal secretion from the circulatory system in 14 experiments performed on 12 anesthetized (Dial) dogs did not prevent the oliguria that usually occurs during the anoxia resulting from the inhalation, through the cannulated trachea, of low oxygen-high nitrogen gas mixtures. The oxygen concentration varied from 6 to 12 per cent. Urine rates of each kidney were recorded continuously from cannulated ureters. The adrenal secretion was prevented from getting into the circulation by occluding the veins approaching and leaving the glands.

Seventeen additional experiments were performed on 17 anesthetized dogs in which one kidney was acutely denervated. The urinary response of the normal kidney to anoxia was the oliguria that occurred in the first series of experiments, the oliguria being independent of changes in the carotid blood pressure. In contrast to this the response of the denervated kidney to the anoxia varied directly with changes in the carotid blood pressure. The elimination of the adrenal glands again did not alter the responses of the normal and denervated kidneys during the anoxia. Apparently the renal nerve supply is the primary factor affecting the urinary responses during the anoxia.

Depletion and regeneration of serum proteins in marine elasmobranchs and teleosts. ABBY H. TURNER. Woods Hole Oceanographic Institution, Woods Hole, Mass. and Mount Holyoke College, South Hadley, Mass.

In continuing a study of serum proteins in fishes, a series of experiments was carried out on the elasmobranch *Mustelus canis*, smooth dogfish, and on the teleost, *Tautoga onitis*, tautog. Individuals were bled severely and repeatedly. Of 18 dogfish, 9 withstood these hemorrhages from 3 to 5 times. Of 16 tautog, 6 were bled from 3 to 7 times. One *Prionotus carolinus*, sea-robin, was bled 4 times. The determinations made on the serum were colloid osmotic pressure, protein nitrogen (analyses by Helen Murphy), refractive index of colloid fraction, with also approximate percentage of corpuscles, and, in about half the specimens, muscle water. The results were studied for evidence of reserve supplies which maintain the protein level, for power of protein regeneration, and for relation of colloid osmotic pressure to muscle water.

Though individuals varied, both species showed a tendency to main-

tain the protein level through two or sometimes three hemorrhages. In the tautog, protein nitrogen was better maintained than colloid osmotic pressure, thus indicating a shift to larger protein particles. In one elasmobranch and two teleosts marked protein regeneration occurred after a pause in bleedings with abundant feeding. In this dogfish all protein values showed regeneration, though elasmobranch c.o.p. is always low. The c.o.p. in these teleosts continued its fall, thus emphasizing the importance of protein content rather than osmotic level.

Both species withstood without observable edema a c.o.p. as low as one fourth the initial value. That the colloid osmotic pressure is, however, not without significance in controlling fluid balance between blood and tissues was shown in both species by a definite though not conspicuous rise in muscle water coincident with lowered c.o.p.

The two-fold value of serum proteins thus appears evident in fishes as in higher groups. The low elasmobranch level of c.o.p. (37 mm. H_2O , average for dogfish) indicates less emphasis on this protein characteristic than in teleosts (115 mm. H_2O , average for tautog). The tendency to maintain the serum protein content evident in both species indicates a relation between serum proteins and the protein nutritive cycle.

Toxicity and pharmacology of vitamin B₆. KLAUS UNNA (introduced by Hans Molitor). Merck Institute of Therapeutic Research, Rahway, N. J.

A study has been made of the toxicity and pharmacology of vitamin B₆ (2-methyl-3-hydroxy-4, 5-bis(hydroxy-methyl) pyridine, recently synthesized by Harris and Folkers) on animals maintained on complete diets.

Acute toxicity studies in rats showed the L. D. 50 to be 3.1 grams per kilogram following subcutaneous and 4 grams following oral administration. Doses up to 1 gram were tolerated without untoward effects. Higher doses produced rather peculiar delayed toxic manifestations. Two days after dosing the animals showed convulsions of a clonic type with the hindlimbs rigidly extended and the forelimbs bent under the body. The righting reflexes were markedly impaired. Between attacks the animals were able to crawl and to take some food. Animals which survived these toxic doses exhibited convulsive seizures for two or three weeks. The toxic manifestations suggest involvement of certain parts of the nervous system. Histologic investigations are in progress.

Chronic toxicity was studied in rats, dogs and monkeys by daily feeding of doses up to 10 mgm. per kilogram over periods extending to 3 months. No significant differences in weight nor in the hemoglobin, erythrocytes, leucocytes and differential blood counts from control animals were found. Rats receiving 2.5 mgm. daily were raised through the third generation with no impairment of growth or reproduction.

Pharmacology. Twenty milligrams per kilogram injected intravenously into cats had no effect on either the blood pressure or the respiration. Isolated organs (uterus and intestine of rabbits) likewise were not influenced by vitamin B₆ in solutions up to 1 to 10,000.

The basal and water metabolism of normal rats was not influenced by doses of 100 mgm. per kilogram.

To determine whether B₆ is excreted normally, biological assays with the urine from men, dogs and rats maintained on adequate diets were made, using B₆ depleted rats as a test object. Less than 0.5 microgram

of B_6 per cubic centimeter were found in the urine of men and dogs. The urine of rats contained B_6 in amounts from 0.5 to 1.0 microgram per cubic centimeter.

The purification of pituitary gonadotropic hormones. H. B. VAN DYKE, R. O. GREEP (by invitation) and BACON F. CHOW (by invitation). Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick, N. J.

The purification of pituitary gonadotropic hormones (hog and sheep) was continued from a stage already described (J. Endocrinol. **1**: no. 4). Every possible precaution to avoid denaturation of protein was taken at each stage. Solutions of ammonium sulfate, sodium sulfate or potassium acid phthalate, adjusted to an appropriate pH, were found most useful as solvents or precipitating agents. Gonadotropic hormones after the initial fractionation were soluble in 0.5 saturated ammonium sulfate at pH 4.2 whereas the precipitate contained no gonadotropic hormone. It is believed that the concentration of protein (initially equivalent to 4 mgm. of N per cc.) is important at this stage. Attempts to fractionate further the gonadotropic hormones were made after removal of ammonium sulfate by dialysis. The most successful solvent contained 0.25 M acetate buffer, pH 4.4, and 20.5 per cent sodium sulfate—although it appeared that considerable latitude in the concentration of sodium sulfate is permissible. The purity of the fractions obtained is believed to be of high degree on the basis of 1, solubility characteristics, and 2, biological activities in immature hypophysectomized male and female rats.

The effect of noise on gastric secretion. EDWARD J. VAN LIERE, PAUL E. VAUGHAN (by invitation) and DAVID W. NORTHUP. Department of Physiology, School of Medicine, West Virginia University, Morgantown.

Four dogs with Pavlov pouches were maintained on a diet of white bread, ground beef heart, and milk. They were placed in stocks and the basal secretion collected for one hour. They were fed a standard meal of 250 grams of beef heart which had been brought to a boil in 250 cc. of water and allowed to cool. Samples of gastric juice were collected every half hour for 5 hours after feeding and were measured for volume, concentration of free and total acid, total chlorides, and for pH.

After the control data had been collected, the animals were subjected to 100 decibels of noise (measured by a sound-level meter) which was produced by an audio signal generator and amplifiers. The 100 decibels of noise was the total noise made up from the noise of the loud speakers superimposed on a background level of 30 decibels at which the control experiments were performed. The noise was continuous throughout the experiment. Five experiments on each dog were performed using a frequency of 600 cycles and later, five using a frequency of 2,000. All experiments were performed in the same room.

In the case of volume and total acid, the data were investigated from the point of view of the total amount secreted in the two and one-half hours immediately following feeding. Volume was expressed in cc. and the acid in milligrams of HCl. At a frequency of 600, one dog showed a significant reduction in the volume of gastric juice secreted. None showed a significant reduction in acid. Neither volume nor acid in the average of the four dogs was significantly affected at a frequency of 600.

With a noise frequency of 2,000, two dogs showed a significant reduction

in volume and the same two dogs showed a reduction in acid. The average of all four dogs showed a significant reduction in both volume and acid at a frequency of 2,000. We conclude that the higher pitch noises are of greater depressing effect and that variation between individuals is of great importance.

Maintenance of the habitus of pregnancy and timely onset of labor after removal of the fetus. G. VAN WAGENEN and W. H. NEWTON (by invitation). Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Conn., and Department of Physiology, University College, London. (Read by title.)

The availability of breeding monkeys with known conception dates presented the opportunity to study pregnancy in a primate. Investigations concerning the role played by the retained placenta in lower animals have been extended to the rhesus monkey, an animal in which gestation covers months rather than days and in which the uterus, placentation and type of menstrual cycle is similar to the human. Pregnancy duration in this monkey is given by Hartman as 6 lunar months or 168 days. In our colony living babies have been born after gestation periods ranging from 152 to 180 days.

When mouse fetuses were removed on the ninth to thirteenth days of gestation (Selye, Collip, Thomson, 1935; Newton, 1935) the placentae remained in place and apparently functional until expulsion on the twenty-first day, the normal time of parturition.

To study the physiology of the monkey with a retained placenta eleven animals were mated and the estimated day of conception counted as day one of gestation. Fetuses were removed at different intervals, as early as the 70th and as late as 157th day. In ten of these monkeys the placenta remained *in situ* and functional until the cervix dilated and contractions of the uterus caused spontaneous expulsion of the placenta at a time when a living baby might have been expected.

Removal of the fetus was usually followed by a period of weight loss due to dehydration and trauma of caesarean section after which the weight increased until an incline parallel to that of early pregnancy was assumed. This reestablishment of pregnancy weight increment was not found following operation toward the end of pregnancy. Beside the weight increase the animals maintained the appearance of pregnancy by a generalized edema, intensification of the sex skin color and physical inactivity. Lactation did not follow excision of the fetus.

It was concluded that the habitus of pregnancy was supported by the retained placenta and that the onset of labor was independent of the presence of the fetus.

The determination of the volume of ellipsoidal erythrocytes by an electrical conductance method and the effect of streaming orientation. SIDNEY VELICK and MANUEL GORIN (introduced by Eric Ponder). Department of Protozoology, Johns Hopkins School of Hygiene and Public Health, Baltimore, and the Biological Laboratory, Cold Spring Harbor, Long Island, N. Y.

The electrical conductance of a colloidal suspension is a function of the individual conductances of the particles and suspending medium and of the fraction of the volume occupied by the particles, their shape and orien-

tation, and at high concentrations, their array. Electrical conductance measurements of suspensions can thus provide information concerning the volume, shape, and conductance of the suspended particles. The existing theory, limited to suspensions of randomly oriented spheroids has been extended to the more general case of ellipsoids with three axes different. The method was applied to avian erythrocytes and gave values two percent below that calculated from axial measurements taken from calibrated microphotographs, assuming a perfect ellipsoidal shape. Centrifugation at 14,000 r.p.m. for eight minutes gave hematocrit values three to four percent above those obtained by the electrical method. Large fluctuations after stirring seemed to be due to flow orientation of the asymmetric cells. The theory was extended to cover three types of orientation and tested experimentally in conductivity cells in which the blood flowed continuously between the electrodes. The theoretical predictions were confirmed. The occurrence of orientation was verified by light transmission experiments.

Studies of osmotic activity of fluids undergoing absorption in the intestine.

M. B. VISSCHER and R. R. ROEPKE (by invitation). University of Minnesota, Minneapolis.

Using the thermocouple method of measuring vapour tension it has been found that the osmotic activity of originally isotonic solutions changes in characteristic and regular ways during absorption. Equiosmotic isotonic mixtures of NaCl and Na₂SO₄ regularly become hypotonic, in some cases to the extent of a half atmosphere osmotic pressure during the early absorption period. The animal's own blood serum placed in the intestinal loop likewise becomes strongly hypotonic. Na₂SO₄ solutions tend to approach the osmotic activity of the blood. Poisoning by 0.001 M HgCl₂ abolishes the decrease in osmotic activity in the cases of mixtures of NaCl and Na₂SO₄ and of serum, but causes Na₂SO₄ solutions to become hypertonic. Analyses of $[\bar{Cl}]$ and $[\bar{SO}_4]$ show that poisoning increases the rate of absorption of the latter and decreases the rate for the former, when present singly as sodium salts in isosmotic solutions. The hypertonicity produced during absorption of Na₂SO₄ from poisoned loops is due mainly to entrance of NaCl into the gut fluid.

The results are in general harmony with the view that the normal ileal epithelium is relatively impermeable to \bar{SO}_4 , and actively moves NaCl into the blood. Both the selective impermeability and active absorption are reduced or abolished by HgCl₂.

*Some pharmacologic effects of N-propyl theobromine.*¹ E. VOGT (by invitation) and R. A. WOODBURY. Department of Physiology and Pharmacology, University of Georgia, School of Medicine.

The acute effects of N-Propyl theobromine have been determined upon the cardio-vascular system, coronary system, intestinal and renal volumes, intestinal movements, urinary output, respiratory movements and reflexes. Cats, dogs and rabbits were used.

Intravenous injections cause at first a fall in blood pressure followed by a prompt recovery. The heart rate is increased. Toxic doses produce pulsus alternans. On the isolated heart, N-Propyl theobromine, in proper

¹ This work was aided by a grant from the American Medical Association.

concentration, increases the coronary flow, and, at times, the force and rate of cardiac contraction. Independently of blood pressure changes, respiration is either markedly or moderately stimulated, not depressed. Intestinal movements are increased. Intestinal volume is increased; kidney volume changes are apparently passive.

Convulsions are sometimes produced by moderate doses. Reflexes in the spinal cat are definitely increased and tetanic convulsions often result.

Acknowledgement is gratefully made to Dr. E. C. Kleiderer of the Eli Lilly Research Laboratories for preparing the drug for us.

The composition of dolphin milk. B. J. VOS, JR. (by invitation), LILLIAN EICHELBERGER (by invitation), E. S. FETTER, JR. (by invitation) and E. M. K. GEILING. Department of Pharmacology, Lasker Foundation for Medical Research and the Departments of Medicine and Physiology of the University of Chicago, Chicago, Ill.

Data will be presented on the major constituents of milk obtained from three live dolphins (*Tursiops truncatus*). In addition, a post mortem sample of milk was secured from a spotted dolphin one and one-half hours after harpooning. The results reported are significant because they were obtained chiefly from uncontaminated specimens drawn from live animals.

The points of interest in the data are: 1, the fluids are rich in fat and protein, and 2, low in lactose; 3, the sum of the inorganic base concentrations ranges from 120-130 m-eq. per liter, except for the animal approaching the end of lactation when the total base is 148.6 m-eq. per liter. The physiological significance of these results will be discussed.

The effect of various agents on the blood pressures of renal hypertensive dogs.

G. E. WAKERLIN, WALTER GAINES (by invitation), and S. D. MOSNY (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago.

Following bilateral constriction of the renal arteries, the hypertensive blood pressure was permitted to stabilize for 3 to 4 months. Mean blood pressure readings were then obtained from the femoral artery three times a week for one or two months, the substance being studied was administered daily for one month in most cases, and subsequently the pressure readings were continued during another control period of one or two months. Parallel studies were conducted on the blood pressures of normal dogs in some instances.

One hypertensive and one normal dog were given daily subcutaneous injections of dog renin (equivalent in acute pressor effect to 4 units of pituitrin) for two and one-half months. A similar pair of dogs received daily intravenous injections of dog renin for one month. The two hypertensive dogs showed average increases of 10 and 20 mm. Hg respectively in their blood pressures during the injection periods. Each of the normal dogs likewise showed a temporary average increase of 10 mm. Hg.

One hypertensive and one normal dog received 250 Gm. of fresh hog kidney by mouth daily for one month without any effect on blood pressure.

Adrenal cortical extract, 0.1 cc. per kilo, was given subcutaneously daily for one month to one hypertensive and one normal dog without influencing the blood pressures.

Daily intramuscular injections of 0.1 mgm. per kilo of estrone (one hypertensive dog) and of 2.5 mgm. per kilo of testosterone (one hypertensive dog) for one month were also ineffective.

Pituitrin in a daily dosage of 10 units/kilo intramuscularly to two hypertensive dogs for two weeks did not significantly alter the blood pressures.

Extracts of liver, pancreas, mammary gland, and garlic and parsley, for which therapeutic claims in human essential hypertension have been made, were ineffective even in large dosages.

As others have noted, distemper in four hypertensive dogs produced a prompt drop in the blood pressures to normal or subnormal levels. However, an extensive, mixed streptococcus and staphylococcus cellulitis in two hypertensive dogs did not lower their blood pressures.

Renin tachyphylaxis. G. E. WAKERLIN and C. A. JOHNSON (by invitation). Departments of Physiology and Physiological Chemistry, College of Medicine, University of Illinois, Chicago. (Read by title.)

In general, our observations on tachyphylaxis in dogs due to repeated intravenous injections of crude renin, confirm those of others. The development of tachyphylaxis is not essentially influenced by the presence or absence of anesthesia, type of anesthetic, nephrectomy, or method of preparation of renin. Allowing for individual differences in dogs, the appearance of tachyphylaxis is facilitated by large doses of renin and by short intervals between successive injections.

Tachyphylaxis was produced by dog, hog, and rabbit renins. Of 28 unanesthetized dogs which were rendered completely tachyphylactic to dog renin, 25 proved to be tolerant also to the pressor effect of approximately equal doses of rabbit renin. The other 3 animals, however, although tachyphylactic to dog renin, gave normal or nearly normal pressor responses to intravenous injections of rabbit renin. No explanation is at present available for this observation, although it casts doubt upon the hypothesis that tachyphylaxis results from the exhaustion of renin activator.

The effects of pitressin on the circulation of the blood in the kidney and on urine formation. K. G. WAKIM (by invitation), J. F. HERRICK, E. J. BALDES and F. C. MANN. Divisions of Experimental Medicine and Biophysics, The Mayo Clinic and Mayo Foundation, Rochester, Minn.

By the method of transillumination, observations were made on the effect of pitressin on the glomerular tuft of capillaries and other vessels in the kidney of the frog under urethane anesthesia. Solutions of pitressin of known concentration were applied directly to the illuminated area or injected into the lymph sac or the anterior abdominal vein. Depending upon the quantity used, pitressin produced cessation of the circulation in the glomerular tuft of capillaries for a fraction of a minute to several minutes, and slowed the circulation in the other vessels to such an extent that no axial stream was observable; often the white and red corpuscles could be easily distinguished in the slowly moving stream within the lumen of the vessel.

Simultaneous records of urine flow, renal blood flow, and blood pressure were made on two groups of dogs, one under nembutal anesthesia and the other under chloralosan. It was observed that pitressin injected subcutaneously caused no changes in urine flow, renal blood flow, or blood pressure; while intramuscular injections produced variable but slight changes. However, when given intravenously, pitressin always produced a transient anuria followed by oliguria for several minutes and a marked decrease in

renal blood flow, which did not return to the preinjection value for thirty minutes to one hour. Intravenous injections of pitressin produced a sudden rise in blood pressure followed by a transient fall and then a gradual prolonged, moderate rise which lasted for approximately fifteen minutes.

Observations on renal blood flow before and after the administration of pitressin were made on a group of trained dogs. The results were practically the same as in the anesthetized groups except the decrease in renal blood flow after intravenous administration was greater and lasted longer in the trained animals. In some experiments the initial decrease in renal blood flow was followed by a prolonged and marked increase.

*Further studies on the metabolism of rabbit bone marrow in serum: the metabolism in relation to the marrow histology.*¹ CHARLES O. WARREN, JR. (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York City.

In these meetings a year ago, the author described methods for the study of the respiration, aerobic and anaerobic glycolysis of rabbit bone marrow slices suspended in serum and showed that in this medium the marrow has a much higher and more steady metabolism than in Ringer solutions. Moreover, the metabolism in serum is of a normal type rather than a tumour type suggested by other authors on the basis of studies conducted in Ringer solutions.

In the present paper, these methods are applied to a reinvestigation of the problem of the relation of the metabolism of the marrow to its cellular composition. Three types of marrows are studied: 1, normal marrows; 2, those predominately erythroid, and 3, those predominately myeloid. The erythroid marrows are obtained in two ways: by bleeding the animals and reinjecting the hemolyzed blood intraperitoneally, and by the use of phenyl hydrazine. The myeloid marrows are obtained by repeated injection of sterile saline intraperitoneally and removal of the exudates so induced. In addition to the metabolic studies, cell counts are made from marrow smears in each case and an index is obtained not only of the myeloid-erythroid ratio but also of the relative maturity of the cells in each class. It is found that the metabolism of the young erythroid cells is predominately oxidative, while that of the myeloid cells is predominately glycolytic, thus confirming and extending similar results based on studies made in Ringer solutions.

Protective effects of various anions and cations against the toxic systemic (particularly convulsant) actions of local anesthetics. H. WASTL. Department of Anatomy, Hahnemann Medical College, Philadelphia, Pa.

The general toxic systemic effects of local anesthetics (procaine, butyn, pontocaine) were studied (guinea-pig intramuscular injections) when given alone and in conjunction with various inorganic and organic salts: halogen salts, benzoates, salicylates, levulinates, gluconates, lactates, acetates.

The following examples (results of some 500 experiments) may illustrate the trend. Butyn sulfate (25 mgm./kgm.) produced in 100 per cent violent convulsions and opisthotonus, beginning in 6 minutes and lasting 20 minutes with death in 42.5 per cent. The same butyn dosage plus levulin-

¹ Supported by a grant from the Committee on Scientific Research of the American Medical Association.

ates added either to the solution or injected separately immediately before gave the following results: with Ca levulinate (300 mgm/kgm.) in mixture—no reaction; separately—37.5 per cent convulsions, less violent, beginning in 10 minutes, lasting 20 minutes, mortality 29.2 per cent. With K levulinate (300 mgm/kgm.) in mixture—25.0 per cent convulsions, less violent, beginning in 9 minutes, lasting 11 minutes; separately—29.2 per cent convulsions, starting in 15 minutes, lasting 18 minutes. With Na levulinate (300 mgm/kgm.) in mixture—47.7 per cent convulsions, beginning in 13 minutes, lasting 18 minutes; separately—12.5 per cent convulsions, very much more less violent, starting in 15 minutes, continuing for 20 minutes. No death occurred with these latter two salts. With CaCl_2 (100 mgm/kgm.) in mixture—37.5 per cent convulsions beginning in 5 minutes, lasting 13 minutes, no death; separately—100 per cent convulsions, starting in 6 minutes, lasting 20 minutes, 42 per cent mortality. KCl and NaCl cannot be used in mixture due to precipitation. Given separately, they do not affect the percentage of convulsions, but do lower the duration and mortality.

Similar results were obtained with a number of dosages of the three local anesthetics tested in combinations with various concentrations of the above mentioned salts in their Ca, Na and K forms. The work (to date over 9000 experiments) shows the relative protective effects of the salts on the main site of action of the local anesthetics (i.e., central nervous system) in a sliding scale, based on the anion constitution. The cation effect shows itself in the local actions.

Pressure-soluble products in monolayers from normal and digested proteins.

DAVID F. WAUGH and IRVING LANGMUIR (introduced by F. O. Schmitt).

General Electric Company Research Laboratory, Schenectady, N. Y.

After compression to 25 dynes/cm. for several minutes and re-expansion to 1 dyne/cm. protein monolayers exhibit characteristic permanent decreases in area as follows: Egg albumin 3 per cent, insulin 12 per cent, gliadin 12 per cent, pepsin (crystalline) 1 per cent, trypsinogen (crystalline) 6 per cent. These permanent area decreases are due to the forcing of film molecules (protein impurities or degradation products) into the substrate by the pressure. Additional compressions and expansions, force-time or (F, t) cycles, up to 25 dynes/cm. indicate no further pressure solubility.

Standardized (F, t) cycles subsequent to the first yield reproducible force-area curves in the form of closed loops (F, a) loops, which differ greatly between proteins.

Compressions above 35 dynes/cm., subsequent to an initial cycle up to 25 30 dynes/cm., cause additional permanent decreases in area at 1 dyne/cm. which are an exponential function of the pressure. (F, a) loops, above 35 dynes/cm., may now be almost reproducible. Such permanent decreases in area produced by compressions above 35 dynes/cm. cannot, however, be correlated with the appearance of soluble products in the substrate and are therefore due to a redistribution of film material. Any permanent decreases in area after compression have generally been associated with collapse. We define collapse, however, as any permanent decrease in area not accompanied by the appearance of soluble products in the substrate.

Impurities or degradation products go into solution under pressure and cause permanent decreases in the specific area at 1 dyne/cm. This sug-

gested that the film technique might be used to follow the progress of protein degradation. Digestion of 2 per cent insulin at pH 2.6 with crystalline pepsin has been examined. At intervals during the digestion period a film is spread and the decreases in specific area at 1 dyne/cm. after successive compressions to 5, 10, 15, 20 and 25 dynes/cm. for 10 min. each are obtained. Such results, which show that the character of the degradation products changes since they are driven into solution by progressively lower pressures, will be discussed.

*Production of fibrillation by alternating current.*¹ RENE WEGRIA (by invitation) and CARL J. WIGGERS. Department of Physiology, Western Reserve University Medical School, Cleveland, O.

With our previous demonstration (Am. J. Physiol., 1940) that brief, strong localized shocks of different forms cause ventricular fibrillation only if they are applied during late systole, the suggestion was made that danger of fibrillation from prolonged direct current is related to their closure or opening during such vulnerable periods. The present report is an initial step in analyzing the mechanisms by which alternating current induces ventricular fibrillation.

In dogs, under sodium barbital anesthesia, direct localized stimulation of a ventricle through nonpolarizable stigmatic electrodes by a 60-cycle alternating current of different duration and strength, may produce: 1, no response; 2, one ventricular extrasystole; 3, two or—rare occurrence—a few ventricular extrasystoles; 4, a few ventricular extrasystoles followed by ventricular fibrillation.

a. Short series of sine waves (2 to 5 cycles) when they start in or fall entirely *in the vulnerable period* always give a response if strong enough; that response is one or two ventricular extrasystoles followed or not by ventricular fibrillation. The experiments have not been sufficiently refined to establish any influence of the moment of onset or phase angle in relation to the vulnerable period. A similar stimulus *starting before* the vulnerable period produces extrasystoles and fibrillation less often. When given *in diastole*, it causes one extrasystole and *never* produces ventricular fibrillation.

b. Longer series (8 to 14 cycles—too long to fit into the vulnerable period only) when starting *before* but extending into the vulnerable period, may not give any response but generally causes extrasystoles or ventricular fibrillation. When starting *during* the vulnerable period, they always produce either extrasystoles or fibrillation. When they are entirely in diastole, they may produce one or two extrasystoles but also fibrillation. The fact that this does not occur with a short series suggests that some of the late sine waves fall during the vulnerable period of a premature systole evoked by earlier waves of the train.

Effect of medial lemniscus section on weight discrimination. E. WEINSTEIN (by invitation), O. SJÖQVIST (by invitation) and J. F. FULTON. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.²

Section of the medial lemniscus was performed in eight macaques and

¹ Aided by a grant from the Markle Foundation.

² This investigation was aided by grants from the Abrahamson Foundation, Rockefeller Foundation and Fluid Research Funds, Yale University School of Medicine.

two chimpanzees. The loss of proprioceptive function and subsequent recovery were studied neurologically and in the chimpanzees by training in weight discrimination. After section of the mesial fillet at the level of the inferior colliculus relatively little residual defect was observable. In the chimpanzee the loss of weight discrimination after thorough retraining approximates (85 per cent correct at a ratio of 1:1.21) that found by Ruch, Kasdon and Fulton (these abstracts) after ablation of the parietal lobe. Section of the opposite brachium conjunctivum after previous interruption of the medial lemniscus adds considerably to the impairment of weight discriminatory ability. Primary lesion of the superior cerebellar peduncle produced no detectable deficit in discrimination of weight in the monkey.

It is suggested that the dentato-thalamic projection to the motor cortex is concerned with the recognition of weight differences and that this system of fibers may subserve the recovery of sensory function following parietal lesions.

Development and persistence of fundamental motor patterns in the absence of sensory control. PAUL WEISS. Department of Zoology, The University of Chicago, Chicago, Ill. (Motion picture demonstration.)

The motion pictures demonstrate: 1, the persistence of motor coordination and tonus in de-afferented limbs of the adult toad; 2, motor patterns in frog larvae after *a*, complete de-afferentation of trunk; *b*, transection of all spinal commissures; *c*, combinations of operations *a* and *b*; 3, the autonomous appearance of motor patterns in the developing hind limbs of the frog in the absence of sensory innervation; 4, motor coordination in de-afferented hind limbs of the rat.

Unmodifiability of muscular coordination in the rat, demonstrated by muscle transposition and nerve crossing. PAUL WEISS and ROGER W. SPERRY (by invitation). Department of Zoology, The University of Chicago, Chicago, Ill. (Motion picture demonstration.)

The motion pictures illustrate the functional performance of rats with hind legs operated as follows: 1, tendons of plantar flexors and dorsi-flexors of foot crossed, other shank muscles excised; 2, nerves of plantar flexors and dorsi-flexors crossed; 3, operations 1 and 2 combined. Operation 1 produced reversal of all foot movements, both reflex and "spontaneous," without any sign of "reeducation" even after prolonged training (over one year). Operation 2 had similar effects, the transposed nerves obviously having passed the phase of reversible "modulation" (P. Weiss). Operation 3, involving double reversal of sense of foot movements, led again to correct use of limb.

The digestibility of meats. J. A. WELLS and M. M. POMARANC (introduced by John Gray). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The present study was undertaken to determine which meat, chicken, old fashioned or tenderized ham, is most readily digested.

In order to answer these questions we have resorted to *in vitro* digestion, human gastric digestion, and digestion in the experimental animal. The *in vitro* digestion studies were carried out by using a digestion mixture containing 0.5 per cent pepsin—0.38 per cent HCl. The conditions of temperature, pH, and degree of agitation were controlled. The human

gastric digestion studies were performed on 8 subjects, using 3 determinations on each subject for each type of meat. The method used was to feed meat equivalents of 4.458 grams of N, and to remove the entire stomach contents at the end of 1 hour. Precipitation with 4 per cent trichloroacetic acid was accomplished on the gastric contents to separate the digested from the undigested residue. In the studies on the experimental animal, determination of the coefficients of digestibility of meats, supplemented to the diet of the depancreatized animal were used.

The results of the *in vitro* digestion studies show: Tenderized ham is 64.27 per cent digested at 1 hour, chicken 70.06 per cent, and old fashioned ham 71.35 per cent. No significant difference exists between chicken and old fashioned ham, both being more readily digested than tenderized ham. The results of the human digestion studies show: 16.82 per cent of the chicken was recovered at 1 hour, 19.31 per cent of the old fashioned ham, and 27.02 per cent of the tenderized ham. There is no significant difference between the old fashioned ham and the chicken, both leaving a significantly smaller residue than tenderized ham. The results of the studies on the experimental animals show coefficients of digestibilities as follows: supplemented old fashioned ham, 61.53 per cent, tenderized ham, 61.63 per cent, and chicken, 56.62 per cent; these differences are not significant. These unexpected results will be explained.

*Effect of gastric filling on antral and bulbar pressure patterns.*¹ JACOB M. WERLE (by invitation), DANIEL BRODY (by invitation) and J. P. QUIGLEY. Department of Physiology, Western Reserve University Medical School, Cleveland, O.

The optical registration method of Meschan, Brody and Quigley was employed to record antral and bulbar intralumen pressures of trained dogs.

During fasting, antral and bulbar pressures during periods of *quiescence* show basal pressures of 0 to 3 cm. water. They rise simultaneously during inspiration to 5 cm. and fall with expiration to the basal level. Similar basal pressures obtain in the *active* empty bulb but basal antral pressures, though roughly parallel are 0.5 to 1 cm. higher. Periodic augmentation of pressure occurs in both regions approximately four to six times per minute. Typically, the antral pressure wave precedes the bulbar wave slightly, persists for 5 to 7 seconds and reaches a maximal pressure of 15 to 30 cm. The bulbar wave persists for 2 to 3 seconds and reaches a maximum of 15 to 30 cm. Occasionally, antral or bulbar pressures of 60 to 90 cm. occur. Periods of activity continuing for 3 to 5 minutes alternate with rest periods of 30 to 90 minutes duration.

When 100 cc. of strained corn meal and meat mush is administered orally, the normal antral and bulbar pressure variations are replaced by changes of 0.5 cm. magnitude occurring 30 to 50 times per minute. At the cessation of deglutition the normal antral and bulbar pressure pattern is promptly regained. An identical record is obtained if food is simply placed near the animal's nose. However, restoration of the normal pressure pattern usually occurs two minutes following removal of the olfactory stimulus. This action of eating or smelling food is equally effective in the full or empty stomach.

No immediate change in antral or bulbar pressure follows the oral in-

¹ This investigation was aided by a grant from the American Medical Association.

gestion of mush, but after 1 to 5 minutes the pressure reaches 4 to 7 cm. in the antrum and 1 to 3 cm. in the bulb and pressure waves become more frequent. However, introduction of 100 cc. of mush *via* the gastric cannula immediately elevates antral pressure to 3 to 4 cm., and this basal pressure persists for approximately an hour. The contrasting response to material administered orally or by cannula is likewise obtained from vagotomized dogs.

*The "androgen" excretion of certain cancerous and non-cancerous individuals.*¹

NICHOLAS T. WERTHESSEN (by invitation) and GREGORY PINCUS. Endocrine Laboratory of the Joseph H. Pratt Diagnostic Hospital and the Boston Dispensary and the Physiological Laboratories, Clark University, Worcester, Mass.

The "androgen" content of the neutral fractions of the urines of 29 patients was measured by means of the Zimmerman reaction for 17-ketosteroids. Determinations were made before and after the administration of (1) 2.1 mgm. of oestrone and (2) 2.1 mgm. oestrone plus 10 mgm. progesterone. The female patients with cancer had malignancies chiefly of the uterus and cervix, the males chiefly malignancies of the gut. The mean urinary outputs per 48 hours are presented in table 1.

These data are taken to demonstrate: 1, that cancerous individuals tend to excrete less "androgen" than non-cancerous persons; 2, that after oestrogen administration the urinary output of "androgen" increases, but to a lesser extent in cancerous persons; 3, that simultaneous oestrone and progesterone administration increase the "androgen" output slightly. The decreased urinary "androgen" of cancerous individuals may be due to an altered steroid metabolism (cf. G. Pincus and M. Graubard, *Endocrinology*, in press), or to the slight age difference between individuals in the two groups. It is considered unlikely that the increased "androgen" output after oestrone injection is due to conversion of oestrone to "androgen" but rather to the sparing action of oestrone on adrenal steroids (G. Pincus, 1937, Cold Spring Harbor Symposia on Quant. Biol. 5: 44; G. Pincus and N. T. Werthessen, *Am. J. Physiol.* 124: 484, 1938; *Proc. Roy. Soc. B.* 126: 315, 1938).

TABLE 1

TYPE OF PATIENTS	SEX	NUMBER	COLOR UNITS BEFORE HORMONE ADMINISTRATION	COLOR UNITS AFTER INJECTION OF 2.1 MG.M. OESTRONE	COLOR UNITS AFTER INJECTION OF 2.1 MG.M. OESTRONE PLUS 10 MG.M. PROGESTERONE
Non-cancerous	♀	9	109	193	195
Cancerous	♀	13	74	107	148
Non-cancerous	♂	2	162	578	949
Cancerous	♂	5	70	128	108

Observations on diodrast and inulin clearances. H. L. WHITE. Department of Physiology, Washington University School of Medicine, St. Louis, Mo.

¹ Aided by grants from the Ittleson Foundation and the Dazian Foundation for Medical Research.

Using a permanganate digestion method for diodrast, clearances of diodrast and of inulin have been carried out on a number of dogs and human subjects. Work with kidney explant dogs shows that diodrast is not completely cleared, the plasma extraction being 60 to 85 per cent complete. Some diodrast is contributed by the red cells during a renal passage. Plasma diodrast clearance in the dog averages 85 per cent (70-95) of renal plasma flow simultaneously and independently measured. There is evidence that plasma diodrast clearance is a closer measure of renal plasma flow in man than in the dog. Phlorizin depresses diodrast plasma clearance, probably more than can be accounted for on the basis of circulatory changes.

The effects of interrenal ligation of the vena cava on kidney function. J. L. WHITTENBERGER (by invitation) and CHARLES HUGGINS. Department of Surgery, University of Chicago, Chicago, Ill.

In earlier studies it had been found that ligation of the inferior vena cava above the renal veins in dogs was fatal. In the present experiments it was found possible to ligate this vessel between the renal veins so that one kidney was subjected to an increased venous pressure while its mate served as a normal control.

The ureters and a small cuff of bladder mucosa were transplanted into each inguinal region in female dogs enabling quantitative collections of the separate renal secretions to be made. Renal function was studied by creatinine clearances, phenol red excretion, etc. Arterial and venous pressures were obtained manometrically in the femoral vessels.

In acute experiments the venous pressure in the femoral and renal veins were found identical. In the normal animal there were slight or no differences in function in the paired kidneys.

Following interrenal ligation of the cava, cyanosis, slight edema, and a markedly increased venous pressure occurred in the lower part of the body in the first few days. Marked functional depression of the congested kidney, without anuria however, and a compensatory increase of function in the normal kidney were present in the first few days followed by a gradual return of each kidney to normal after several weeks. Following complete recovery the cava was ligated above both kidneys and changes in the kidney function were induced somewhat milder than after interrenal ligation and in the opposite kidneys.

Effect of age on ketosis. ARNE N. WICK (by invitation), EATON M. MAC-KAY and HERBERT O. CARNE (by invitation). The Scripps Metabolic Clinic, La Jolla, Calif. (Read by title.)

Albino rats fasted from a stock diet develop a ketosis as measured by the concentration of acetone bodies in the blood which lasts as long (11 days) as they are fasted. A comparison of the rate of development of this ketosis in male rats 30, 45, 61, 76, 128 and 275 days old shows that the greater the age the more slowly this ketosis develops. At 30 days of age the maximum level of acetone bodies in the blood has almost been reached in 12 hours while the 275 day old rats required nearly 60 hours of fasting for it to be reached. There was no certain influence of age upon the maximum blood acetone body level reached in fasting rats. The level reached was usually between 20 and 25 mgm. per cent. Acetone bodies are not excreted in the urine in measurable quantities at these blood levels. Neither the

tivities on the tetanized root. Facilitation was not observed after antidromic tetani to ventral roots.

The availability of sodium lactate for brain oxidations. JOSEPH WORTIS (by invitation) and WALTER GOLDFARB. Psychiatric Division, Bellevue Hospital, New York City.

The evidence for the oxidation of lactic acid by brain tissue is contradictory at present. *In vitro* studies in the Warburg apparatus indicate that lactic acid may be oxidized as readily as glucose. It also appears that in the presence of fluoride glucose cannot be oxidized, while lactic acid oxidation is not inhibited (Holmes). This would seem to indicate that the oxidation of glucose proceeds only after conversion to lactic acid. Himwich et al., found no evidence of oxidation of lactic acid by the human brain tissue *in vivo*. We have investigated the availability of lactic acid for brain oxidations in human subjects when the amounts of glucose available were minimal, namely during insulin hypoglycemia. We found that the oxygen consumption of the brain did not increase when small amounts of lactate were administered, and only increased moderately with large doses. Oxidations were never sufficiently accelerated to rouse the patients from the insulin coma. It therefore seems probable that during insulin hypoglycemia in humans only small amounts of energy, if any, may be derived from the oxidation of lactate by brain tissue.

*The effects of carotid sinus reflexes on convulsions.*¹ L. YESINICK (by invitation), E. GELLHORN, and C. DARROW. Department of Physiology, College of Medicine, University of Illinois, Chicago. (Read by title.)

Experiments on cats and dogs with chloralose anesthesia (100 mgm. per kilo) involving various convulsive drugs (metrazol, picrotoxin, coriamyrtin, absinth, camphor and strychnine) showed that adrenalin (0.004 to 0.015 mgm. per kilo) inhibits temporarily the convulsions simultaneously with the rise in blood pressure. If, however, the buffer nerves are removed the convulsions are aggravated. That the action is due to effects on the carotid sinus receptors is shown by the following observations: 1. Faradic stimulation of the carotid sinus nerve inhibits convulsions. 2. Tilting the animal with the head downward inhibits convulsions also. 3. The tilting described under 2 aggravates convulsions when the carotid sinus area is denervated and the vagi cut.

Steps in the identification of reflexly produced sympathins. W. B. YOUMANS, H. F. HANEY and K. W. AUMANN (by invitation). Department of Physiology, University of Oregon Medical School, Portland.

Methods have been used which result in reflex production of sympathin from mainly inhibited sources (rectal stimulation) and mainly excited sources (acetylcholine hypotension) in unanesthetized, adrenal demedullated dogs. The effects of the sympathin from each source have been compared quantitatively with the effects of adrenalin and simpler phenylethylamine derivatives on both the denervated heart and the denervated intestine.

Various physiological, physical, and chemical properties of sympathin

¹ Aided by grant from John and Mary R. Markle Foundation.

indicate that it is a phenylethylamine derivative, but that it is not identical to adrenalin. The adrenalin molecule differs from phenylethylamine at four points; two phenolic —OH groups, one alcoholic —OH group, and —CH₃ on the nitrogen atom. Removal of any one of the —OH groups results in a marked or differential lowering of intestine-inhibiting and cardiac-accelerating potency, but removal of the —CH₃ group affects the potency so little that adrenalin and non-methylated adrenalin (arterenol) can not be readily differentiated by the two indicators. Either adrenalin or arterenol could be used to duplicate the effects of the sympathins. The other compounds (synephrin, neosynephrin, and epinine) failed to duplicate quantitatively the effects of sympathin and/or produced additional reactions not caused by sympathin. It is indicated that sympathin is not predominately a phenylethylamine derivative simpler in structure than arterenol. It may be postulated that sympathin is a more complex derivative. However, the results do not appear to be explicable by the "two sympathins" theory (Cannon and Rosenblueth) if, as is indicated, mainly inhibited sources for the sympathin are involved in one case and mainly excited sources in the other. No crucial test of the "two mediators" theory (Bacq; Greer et al.) is involved, but the low potency of arterenol as compared with adrenalin in various adrenergically excited effectors appears to be an obstacle to considering arterenol the mediator rather than a step in its breakdown.

Numerous facts suggest that sympathin may be a mixture of adrenalin, non-methylated adrenalin, and possibly other detoxication products in varying proportions depending on the enzymic activity of the effector concerned and the rate of production of adrenalin, the mediator.

Motility of the human small intestine. W. B. YOUNG, H. F. HANEY, H. P. RUSH (by invitation) and W. ZAVIN (by invitation). Departments of Physiology and Medicine, University of Oregon Medical School, Portland. (Motion picture demonstration.)

A patient having a large defect in the abdominal wall through which numerous coils of the small intestine protruded was available. The herniation occurred over twenty years ago and was protected by a canvas belt during most of that time. The skin covering the hernia has become so thin and loose that it assumes the contour of the intestinal coils when the patient is in the supine position, thus permitting filming of intestinal motility under essentially normal conditions. The patient has had no operation involving the intestine and there have been no symptoms of disturbed intestinal function. Motion pictures obtained during the following experiments will be shown: 1. Effect of taking food following a short fast. 2. Effect of clinical dosage of morphine. 3. Effect of clinical dosage of prostigmin followed by atropine. 4. Effect of clinical dosage of pituitrin. 5. Determination of the threshold intestine-inhibiting injection rate for adrenalin (in gamma per kilogram per minute). 6. Repetition of no. 5 nine days later.

Stickiness of leucocytes and endothelium. BENJAMIN W. ZWEIFACH (introduced by Robert Chambers). Laboratory of Cellular Physiology, New York University, New York City. (Read by title.)

Repeated prodding of the capillary wall or of the nearby tissue produced a transient stickiness, the duration and intensity of which varied according

to the severity of the stimulus. The phenomenon involved three components, the endothelial cells, the inter-cellular cement, and the blood leucocytes. Under normal conditions, stickiness, as evidenced by the adhesion of carbon particles from the blood stream, is restricted to the inter-endothelial cell cement, being most marked on the venous end. The leucocytes likewise showed a more pronounced adhesiveness in the venous portion of the capillary bed. The lack of adhering leucocytes at the arteriolar end is, in part, due to the rapid blood flow. This was demonstrated by compressing an arteriole with a microneedle and thereby slowing the circulation of blood through the vessel, whereupon the leucocytes showed an evanescent sticking to the arteriolar wall but not to the same degree as in the venular end.

The above conditions were disturbed by mechanical irritation with microneedles. Repeated gentle stroking of the vessel produced an augmented sticking of carbon to the cement lines. More localized, prolonged prodding caused the leucocytes to become markedly adhesive and, irrespective of the rate of blood flow, to cling to the capillary wall even in the arterial portion of the network. In addition, the leucocytes not only stick to the vessel wall but to one another, forming long chains of cells. When an adherent leucocyte was prematurely loosened with a microneedle and released into the stream, it became attached to the capillary wall further downstream. In a later stage, carbon tended to adhere all over the endothelial surface, indicating an overall stickiness.

The venous blood could be re-routed by appropriate localized compression so that the blood that had passed through an irritated region, instead of entering a venule, would stream through a capillary in a reverse direction from its normal one. Under these conditions, increased adhesiveness occurred equally as well in the arterial end. This and various other findings lead to the conclusion that the degree of adhesiveness of the endothelium and the leucocytes is related to a release from the irritated region of a substance into the blood stream.

